THE ROLE OF TOMATO AND LYCOPENE CONSUMPTION ON PROSTATE CARCINOGENESIS AND PROSTATE CANCER TREATMENT

BY

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DISSERTATION

Submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Nutritional Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2020

Urbana, Illinois

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Abstract

Prostate cancer (PCa) is most commonly diagnosed cancer among men in the United States. Following PCa diagnosis, men often seek information about foods and supplements that may improve their response to therapies, quality of life and survival. Tomatoes and their primary bioactive (lycopene) are one of the most researched foods that have the potential to reduce prostate carcinogenesis. Currently, the majority of the literature has been observational epidemiological literature with inconsistent results. Although preventing PCa is preferable to treating PCa, patients seeking information about foods and supplements might already have cancer. Importantly, the prostate microenvironment is not the same prior to cancer initiation, during PCa promotion/ progression, and during treatment. As a result, we sought to clarify the epidemiological associations between tomatoes (and lycopene) and PCa incidence, and to evaluate the role of tomato feeding in an animal model that was undergoing two common treatment approaches.

In Chapter 2, we evaluated the associations between tomatoes and PCa incidence. In this meta-analysis, we found that increased tomato consumption was associated with a reduced risk of PCa (RR=0.81, 95% CI =0.71-0.92, p=0.001). This finding was supported by dose-responses for several types of tomato products. In particular, it was observed that bioavailability of lycopene was important. Raw tomato consumption was not associated with a decreased risk of PCa (p=0.487), while cooked tomatoes and sauces (sources with high lycopene bioavailability) were associated with a decreased risk of PCa (p=0.029).

In Chapter 3, we evaluated associations between lycopene and PCa incidence. Similar to the whole food product, increased lycopene consumption and blood concentrations were associated with a decreased risk of PCa. These associations were also supported by dose-response associations. From the dietary meta-analysis in Chapter 2, there was an estimated 9% reduced risk for 100 grams/week of cooked tomato. For an equivalent dose of lycopene (22 mg lycopene in 100 grams of tomato puree according to the USDA Nutrient Database), tomatoes were more effective than lycopene at reducing PCa risk (9% compared to 1.6%). The greater benefit from tomato products may be due to interactions between potentially beneficial bioactive compounds in tomatoes. These meta-analyses support the hypothesis that tomato products or lycopene reduce prostate carcinogenesis.

In Chapter 4, we aimed to determine whether dietary lyophilized tomato powder (TP) or lycopene would be capable of affecting the growth of castration-resistant prostate cancer (CRPC). This aggressive and often lethal stage of PCa occurs after the prostate tumor acquires mutations to sustain growth despite androgen deprivation therapy (ADT). We hypothesized that tomato or lycopene products would reduce the emergence of CRPC. To test this hypothesis, TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mice were castrated at 12-13 weeks of age to model ADT, and the emergence of CRPC was monitored by ultrasound in two studies. In Study 1, TRAMP mice (n=80) were weaned onto an AIN-93G-based control diet (Con-L, n=28), a 10% TP diet (TP-L, 10% lyophilized w/w, n=26), or a control diet followed by a TP diet after castration (TP-Int1, n=26). In Study 2, TRAMP mice (n=85) were randomized onto a control diet with placebo beadlets (Con-Int, n=29), a tomato diet with placebo beadlets (TP-Int2, n=29) or

a control diet with lycopene beadlets (Lyc-Int, n=27) following castration (12 weeks of age). Tumor incidence and growth were monitored by ultrasound beginning at 10 weeks of age. Mice were euthanized 4 weeks after tumor detection or at 30 weeks of age if no tumor was detected. In contrast to studies of *de novo* carcinogenesis in multiple pre-clinical models, tomato components following castration did not reduce CRPC incidence, time to tumor detection or final tumor weight.

In Chapter 5, we hypothesized that dietary TP would not reduce apoptosis or cell death within the prostate tumor following external beam radiation therapy (EBRT) and would protect surrounding tissues from radiation-induced damage. Tomatoes contain carotenoids and other potent antioxidants. To evaluate this hypothesis, we conducted a pilot and dietary study. In the pilot study, male TRAMP mice (n=18) were provided a powdered AIN-93G diet. In the Diet study, male TRAMP mice (n=76) were provided a control diet or a modified AIN-93G diet containing 10% TP (w/w) beginning at 4 weeks of age. In both studies, prostates were monitored by ultrasound for *in vivo* tumor detection and 3-D volumetric measurement biweekly. Once tumors reached a volume of 1000 mm^3 or at 24 weeks of age, the caudal half of the mouse was irradiated with 7.5 gy (Rad) or 0 gy (sham) with a Cobalt-60 source. In the pilot study, mice were euthanized after 0, 24, or 72 hours. Based on the results of the pilot study, mice in the main Diet study were euthanized 24 hours after radiation or sham treatment. Within the Diet study, prostate tumor scores for apoptosis and necrosis were not modified by tomato consumption; however, tomato consumption did not reduce acute changes in radiation-induced inflammation or damage within the prostate and surrounding tissues.

In conclusion, this work has revealed consistent associations between tomato products or lycopene and risk of prostate cancer incidence within published epidemiological studies. In our preclinical studies of tomato products and PCa treatments (ADT and EBRT), it was found that the responses to treatment were not modified by a diet containing 10% TP or lycopene. While further work in animal models and humans are needed, it is notable that these are the first studies to evaluate the interactions between tomato products on PCa treatments. Data from these epidemiological and preclinical studies highlight the importance of healthy dietary patterns throughout the lifespan. These data are very important for patients that are diagnosed with PCa and seek out information about the types of foods or supplements that can improve their clinical outcomes. Further studies should evaluate differences within the tumor microenvironment to determine if tomato products are more effective for specific subtypes or specific clonal populations of PCa. Additional epidemiological studies are also needed to determine if circulating lycopene concentrations are associated with specific stages of PCa and treatment-associated outcomes (such as biochemical recurrence and cachexia). In summary, this dissertation has contributed novel findings to our understanding of the interactions between tomato products and PCa.

Acknowledgements

Throughout my doctoral training, I have been fortunate enough to encounter many scientists, mentors, and friends that have been an excellent source of support, scholarship, and collegiality. First, I would like to thank Dr. John Erdman. Five years ago, I received an email from someone I met during lunch that changed the course of my research career. Interestingly, his research had nothing to do with my interests. I took advantage of the opportunity to meet his students at Experimental Biology in 2015. At this meeting, his students echoed that Dr. Erdman is an excellent mentor but warned that he is a morning person. Five years later, I am very confident in their evaluation and have the same feedback. I am thankful for his guidance, patience, and willingness to train me into the scientist that I am today.

I am also thankful for many of the mentors and advisors that led me to pursue a career as a scientist at University of Missouri – Columbia. During my first year of my undergraduate career, Dr. Brenda Peculis recommended that I explore my intellectual curiosity in a research laboratory. This connected me to Dr. Ulus Atasoy, Dr. Joseph Magee, and Dr. Patsharaporn Techasintana who trained me on many of the methodologies that I use to this day. I am also thankful for Brian Booton and Dr. Mark Hannink who were excellent mentors that taught me about graduate school and were integral to my decision to continue my education in a PhD program. Finally, I am thankful for the guidance of Dr. Catherine Peterson, Dr. Victoria Vieira-Porter, and Dr. Jaume Padilla who were instrumental in the development of my passion for nutrition research.

I also owe sincere gratitude to each of my committee members: Dr. Elizabeth Jeffery, Dr. William O'Brien, Dr. Eric Bolton, and Dr. Jaime Amengual. I am also thankful

for the guidance of Dr. Sharon Donovan and Dr. Rodney Johnson who were members of my annual advisory committees. Each of these committee members have challenged me to think of different aspects of my research and have provided me with insight into new avenues that my research could benefit from. I am also thankful for their advice and recommendations that have aided me in my academic and professional development.

I would like to thank the collaborators that I have had the pleasure of working with during my doctoral research. There is not enough that can be said about the tremendous amount of work that each collaborator has contributed to our studies. Dr. Matthew Wallig has gone above and beyond to evaluate the countless slides for each of our studies and to ensure that we learned through this process as well. Dr. Steven Clinton has also gone above and beyond his role as a collaborator at Ohio State University to provide a critical review of our study designs and their clinical significance. Dr. Clinton has also served as a mentor for me as he assisted in my training in person and by remotely. Dr. Ruopeng An was also instrumental in teaching me about systematic reviews and meta-analyses. His guidance provided the foundation for the statistical evaluation of the literature in Chapters 2 and 3. Dr. William O'Brien, Dr. Rita Miller, Dr. Aiguo Han, and the remainder of the Bioacoustics Research Laboratory provided guidance and insight into several studies. In particular, this collaboration was essential for Chapter 4, Chapter 5, and the completion of 3 other studies that were not presented in this dissertation. In particular, there is not enough that can be said about the role that Dr. Rita Miller had in my training and within the management of the lab spaces at Beckman Institute. Dr. Kimberly Selting, Dr. Timothy Fan, and Dr. Stoyan Toshkov were also collaborators who also went above and beyond to assist in the development and research process for Chapter 5. To this end, we strongly

appreciate Dr. Selting's efforts to keep the Cobolt-60 source active through the completion of the animal work in Chapter 5. Dr. Jesus Sarol, Dr. Salma Musaad, and Amandeep Kaur were extremely helpful and patient with the statistical analysis for Chapter 4. Each of their efforts were instrumental in developing the final results for this section. As Dr. Erdman has stated, "it takes a village, indeed an expanded village" to complete each study that we have conducted.

Each of the members of the Erdman lab, past and present, have provided me with a collegial, collaborative, and enjoyable place to work. I owe a special thanks to Katherine Ranard, Molly Black, Catherine Applegate, and Sookyoung Jeon for their support over the past five years and for their friendship through this process. I am also grateful for the role that Molly Black has served within the Erdman lab. She was integral to the completion of Chapters 4-5 and has been the woman who kept everything running as smoothly in the Erdman lab. I am also thankful for the undergraduate assistance from Jacob White, Elizabeth Nettleton, Ashley Newton, Lindsey Kelso, Edmund Turudic, Michael Cioffi, and Jake Berndt for providing high-quality assistance in our animal studies. In the Division of Nutritional Sciences office, Dr. Jessica Hartke, Dr. Anna Keck, Ashley Browning, Sarah Wells, and Ashley Negangard have all been extremely helpful in navigating through my graduate program.

I would also like to acknowledge the institutions that have funded our research and my graduate studies. The National Institutes of Health, United States Department of Agriculture, Kraft-Heinz, Graduate College, and Division of Nutritional Sciences have all generously invested in our research and my professional development. I am extremely

grateful for each of these programs that have made the research in this document (and many others) possible.

Last, but not least, I am very thankful to my friends and family who have supported me through this process. In particular, I am thankful to Rae McDonald who has been my closest ally and largest supporter throughout my graduate career. To my parents (Masonya and Joe), my sisters (Sasha, Alexandra, and Candace), my grandparents (Joe Rowles, Phyllis Rowles, and Sena Jones) and extended family, I am extremely thankful for the unconditional love and support that you have provided me. I cannot imagine this journey without the love and support that each of you have offered. I am lucky to have had each person that has been a part of this journey.

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Chapter 1: Literature Review

Prostate cancer biology, diagnosis, and treatment

Prostate cancer (PCa) is the second most common cancer among men worldwide and is the fifth leading cause of cancer related deaths worldwide.[1] PCa is a disease of aging.[2] Clinical symptoms are rare in men younger than 50, with approximately 2/3 of cases occurring in men aged 65 and older. Incidence rates vary substantially across countries, primarily due to the advent of prostate specific antigen (PSA) testing.[1,3] For example, PCa accounts for 21% of new cancer diagnoses among men in the United States compared to 15% worldwide.[1,4] Guidelines for PCa screening vary between countries, medical organizations and guideline groups.[5] Differences in screening intervals and guidelines may affect PCa detection and incidence rates.[6] Racial and genetic profiles also modulate risk for PCa.[4,7,8] Although these risk factors are largely based on genetics and location, only 15% of cancers are due to hereditary/genetic mutations. Approximately 85% of cancers are driven by environmental factors that are mostly determined by lifestyle. Diet and nutrition are drivers of nearly 40% of all cancers and modulates risk for PCa.[9,10]

If PCa is detected early, common therapeutic options include: active surveillance, surgery (prostatectomy), external beam radiation therapy (EBRT) or chemotherapy.[11-13] In particular, EBRT has become more precise and novel methods of delivering the radiation dose have been proposed to improve patient outcomes. Despite improved treatment, approximately 60-69% of men who receive EBRT develop biochemical recurrence.[14] The primary limitation for radiation therapy, is the potential for damage to surrounding tissues. Acute and late side-effects on normal surrounding tissues have

limited the amount of radiation that a patient may endure and the therapeutic effectiveness of EBRT. For example, radiation therapy is typically accompanied with bowel and sexual dysfunction,[15] and importantly (but less common), secondary tumors and PCa progression.[16,17] Novel approaches are needed to improve the therapeutic effectiveness of EBRT and outcomes for PCa.

In clinical practice, the maximal dose is typically limited by potential for damage to the surrounding (non-tumor) tissues. As the dose of radiation increases, oxidative damage within the tumor and surrounding tissue increases and the number of required doses (fractions) decrease (Figure 1.1). Acute and chronic toxic effects such as gastroenteritis, bowel dysfunction, sexual dysfunction, reduce the clinical effectiveness of EBRT. A low α/β ratio (1-3 gy range, ~1.8 on average)[18,19] for PCa, indicates that PCa tumors may be highly responsive to lower doses of radiation that are delivered in multiple doses (fractions).[19-21] Hypofractionation is hypothesized to improve tumor free recurrence, reduce biochemical recurrence, reduce treatment-associated trips to the hospital (~50% fewer trips), and reduce cost associated with treatment (~$1900 per patient).[22-24] The surrounding tissues have a higher α/β ratio (~3.6-5 gy for bladder and rectum), which indicates that hypofractionation will not likely affect acute toxicity to normal surrounding tissue. Several clinical trials have investigated the clinical differences between these approaches. All have found that there are no differences between these two treatment

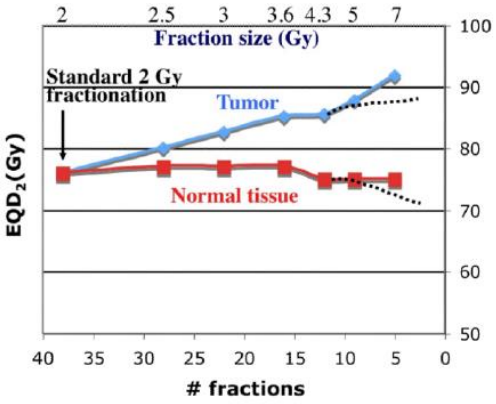


Figure 1.1: Delivery of the total dose for radiation may be achieved through differing fractionation schemes. As fraction size increases, potential damage to the tumor increases.

schemes. This includes toxicity (acute or chronic) and relapse-free survival.[25-30] Pending results from long-term follow up studies, hypofractionation will likely become the next standard of care for PCa treatment.

Despite improvements to PCa detection and treatment, approximately 30% of men treated with curative interventions suffer tumor recurrence.[31] These men, along with men diagnosed with advanced or metastatic cancer, usually undergo androgen deprivation therapy (ADT) to reduce systemic androgens.[31,32] Huggins introduced the first strategies (castration) for reducing serum androgen levels as a treatment for PCa in the 1940s,[33] which led to a Nobel Prize in 1966. Since then newer androgen inhibitors have become safer and more specific toward targeting metabolites along androgen synthetic pathways.[34] Despite these advances and initial success, most patients undergoing ADT progress to an advanced and lethal stage of PCa that responds very poorly to all known therapies. This is a condition known as castration resistant prostate cancer (CRPC).[32,35-37] CRPC is a lethal stage of PCa with a median survival of only 18 months.[38,39] ADT has largely remained the standard for care among men with advanced and metastatic PCa. Recently, chemotherapy and hormonal therapeutic approaches have been integrated into the treatment regiments.[39-42] This led to a 50% increase in survival from 12 months to 18 months. Although progress has been made to improve life expectancy, the prognosis for CRPC is still very poor and accompanied by many negative adverse effects. Advances to improve quality of life and reduce progression of this aggressive stage of PCa are needed.

Tomatoes, carotenoids, and lycopene

Tomatoes are the most consumed non-starchy vegetable in the United States, accounting for 19% of all vegetable consumption.[43] Tomato consumption is generally divided into two forms—fresh tomatoes and processed tomato products. The average intake for tomatoes in America is a quarter of one cup per day, with 56% of typical consumption coming from tomato products and 44% from fresh tomatoes.[43] Tomatoes are good sources of many nutrients and bioactive food components such as lycopene, carotenoids, flavonoids, potassium, folate, fiber, and vitamins that may improve health status.

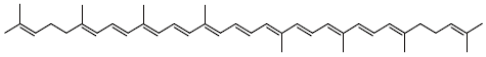


Figure 1.2 Structure of all *trans* lycopene

Lycopene is the dominant carotenoid and primary hypothesized bioactive in tomatoes.[44,45] Carotenoids are a family of compounds of over 600 lipophilic plant pigments that contribute the visible colors of many plants.[44,46] As a carotenoid, lycopene is responsible for the red color of some fruits and vegetables. The structure of carotenoids is based on a C_{40} isoprenoid backbone that may be acyclic or have one or both ends modified into rings. In human sera, lycopene is a mixture of ~50% all-*trans* and *cis* lycopene.[47] All-*trans* lycopene is a linear molecule that is constrained by a system of eleven conjugated double bonds (Figure 1.2). 5-cis lycopene is the second most common isoform in the serum.[48] Of the identified carotenoids, ~60 are found in the diet, with far fewer in detectible quantities in the blood.[46] Dietary sources of each of the most common carotenoids can be found in Appendix A. Feeding synthetic (supplemental) lycopene can also provide lycopene isomers to tissues within a comparable range to the diet, but the whole food

matrix contains additional carotenoids, polyphenols and bioactive components that might be important for disease management.

Lycopene absorption, bioavailability, and metabolism

Before carotenoids are able to be absorbed, they must be released from their food matrix, incorporated into lipid droplets, and then micelles must be taken into the enterocytes.[49] In the duodenum, bile salts reduce the size of the lipid droplet and increase the surface area for lipolytic digestion. Carotenoids that are within the core of micelles may diffuse into the enterocyte.[46,49] In addition to diffusion, lycopene, beta-carotene and lutein may also be actively absorbed through receptor-mediated transport in the duodenum. This transport requires the Class B Scavenger Receptor (SR-B1) membrane protein, which selectively uptakes cholesterol and other lipophilic substances.[50,51] Although SR-B1 has low substrate specificity, beta-carotene is absorbed more efficiently than lycopene through this transport mechanism.[52]

There is a wide intraindividual variation in carotenoid bioavailability. This may be due to a number of factors regarding genetic differences, food preparation procedure, and interactions with other foods during digestion. For example, carotenoids are generally more bioavailable as they are released from their respective food matrix. Food processing, such as heating (cooking/thermal processing), mechanical and enzymatic degradation facilitate the disruption of the cell wall and organelles that contain carotenoids, which allows for dispersal and absorption in the small intestine.[53-55] Because of the highly lipophilic nature of carotenoids, lipid consumption is essential to the adequate absorption of carotenoids. Increased lipid consumption (particularly long chain fatty acids) improves lycopene and carotenoid bioavailability by increasing solubility.[56-58]

Additionally, there is differential absorption of isoforms. *Cis* isoforms of lycopene are generally better absorbed than the all-*trans* isoform.[47] Some soluble fibers disrupt micellization and can reduce the bioavailability of carotenoids. Notably, soluble fibers decrease cholesterol and lipid absorption, which reduces carotenoid bioavailability.[46,49] Genetic variability may play a large role in lycopene bioavailability. In one study, a combination of 28 single nucleotide polymorphisms (SNPs) in 16 genes accounted for 72% of lycopene bioavailability variation.[59] These differences in lycopene bioavailability may affect long-term blood lycopene status.[59,60]

Lycopene is packaged into chylomicrons within the enterocyte and enters circulation.[61] Once in circulation, the half-life for lycopene is 6.2 days (5.3 for *trans*-lycopene and 8.8 days for *cis*-lycopene).[62] Lycopene is deposited in a number of tissues where it can be metabolized. Two enzymes are thought to be involved in the initial cleavage of lycopene. The first enzyme, beta-carotene 15,15'-monooxygenase 1 (BCO1), is cytosolic, has limited substrate specificity, and cleaves at the central bond.[63] It is currently under debate as to whether BCO1 actually cleaves lycopene,[64-67] but BCO1 knockout mice have altered accumulation of lycopene compared to wild type mice.[68] The second enzyme, beta-carotene 15,15'-monooxygenase 2 (BCO2), catalyzes asymmetric cleavage of lycopene to form apo-lycopenals.[69] These apo-lycopenals are also found in the diet, and are absorbed by a similar method to other carotenoids.[70] Apo-lycopenals are metabolically active and may contribute to the potential health benefits in tomatoes.[71,72] BCO2 is located in the mitochondrial matrix and displays specificity for lycopene along with other carotenoids and xanthophylls.[73,74] In BCO2 knockout mice, lycopene is not able to be cleaved and accumulates in the serum, liver, and adipose tissue.[75]

Evaluation of safety for carotenoids

Carotenoid supplementation is well tolerated by most people. Lycopene was assessed in a Phase I-II clinical trial for toxicity with daily supplementation of 15, 30, 45, 60, 90, or 120 mg lycopene (provided as Lyc-O-Mato) per day.[76] Of the 36 patients that were evaluated, one patient developed diarrhea (grade 2 toxicity), and the others did not develop any toxicities attributable to the supplementation. Another study evaluated the observed safety level through a risk assessment method for lycopene.[77] Based on the literature, the observed safety level for lycopene consumption was 75 mg lycopene per day. As a reference, one serving of tomato sauce (half a cup or 120 grams) provides 6.9 mg of lycopene, and an average adult in the United States consumes 4.5 mg lycopene per day. In the same study, lutein was also assessed. The observed safety level in this risk assessment for lutein was 20 mg per day.[77] For reference, one egg yolk has approximately 1 mg of lutein, and an average American adult consumes 1.5 mg of lutein and zeaxanthin per day. Although β-carotene was not directly evaluated for toxicity, it is generally recognized as safe (GRAS, 21CFR, Sect 182.5245, 184.1245) as a supplement and nutrient.[78]

The Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) model

The transgenic adenocarcinoma of the mouse prostate (TRAMP) model was developed in the laboratory of Norman Greenberg during the 1990s. The TRAMP model is a transgenic line of mice that spontaneously developments of PCa in the prostatic epithelium. Prostate epithelium-specific expression of the simian virus 40 large and small T antigen (SV40 Tag) genes are driven by the rat probasin promoter, which leads to

prostate carcinogenesis in all TRAMP mice.[79,80] Probasin expression is androgen dependent and stimulates growth upon puberty in these mice.[81] The viral large T antigen inhibits the tumor suppressors p53 and Rb,[80,82-84] two genes commonly lost in human PCa[39], while the small t antigen inhibits protein phosphatase 2A, a negative regulator of the pro-proliferative MAP kinase pathway.[80,83,84]

This model has been extensively used for investigation of pharmacological and nutritional preventative methods.[79] This model has been used extensively as a model of PCa; at least 1000 original articles appear on PubMed (March, 2020). Primary,[80,85] metastatic,[85,86] and castration-resistant PCa[87-89] have been characterized in this model. Several aspects of the TRAMP model make it an appropriate model of human PCa, especially CRPC. The model demonstrates a progression from early low-grade hyperplasia through to metastasis, similar to human disease.

Castration initially induces prostatic or tumoral regression before emergence of CRPC, and some tumors exhibit AR mutations.[90] One concern with this model has been the presence of neuroendocrine (NE) differentiation. NE markers are indeed expressed in adenocarcinoma lesions of intact TRAMP mice and incidence increases with cancer grade.[89] Tumors in castrated TRAMP mice also contain NE foci,[89] which are not dependent upon circulating androgens.[91] However, it is crucial to recognize that neuroendocrine differentiation in intact and castrated TRAMP mice reflect the pathology of advanced human PCa. NE differentiation is commonly found in many primary human PCa tumors and correlates with both tumor grade and Gleason score.[92] Similarly, NE differentiation is found in human CRPC.[93] Therefore, foci of NE differentiation appear to be a natural function of disease progression in humans and TRAMP mice.

The interaction of diet and cancer is a very complex process that needs to be studied in the whole animal. We are primarily investigating the efficacy of a whole food (tomato powder) in cancer prevention; therefore, an animal model that digests and metabolizes the food and nutrients of interest is critical for any future translation/use for human trials. Animals must be used in order to collect data from serum and tissue analysis, particularly for pathologic tumor grade evaluation. There are no suitable alternatives currently in existence that would provide the unique tissue and cellular environmental and cytoarchitectural features that exist in the TRAMP mouse model that we are proposing to use. This animal model enables a focused and controlled environment for efficient dietary intervention studies to occur.

Diet and the Tumor Microenvironment

Although, tomato and lycopene consumption were found to be inversely associated with PCa progression among observational studies,[94,95] it is not known if this correlation is due to lower overall cancer incidence, interactions with therapeutic approaches, or alterations in the trajectory of disease development. Very few clinical trials have evaluated the effect of lycopene or tomato-based supplementation on PCa progression. In general, the studies have suffered from methodological constraints such as short periods of supplementation and small sample sizes.[96] This has limited the ability to assess the clinical effectiveness of these interventions, creating a knowledge gap regarding the effectiveness of tomatoes or lycopene as a nutritional intervention. While data suggest that tomato consumption may provide a benefit to men with advanced PCa, a lack of convincing preclinical data prevent additional investment in expensive clinical

studies. Ultimately, a better delineation of these interventions may lead to novel nutritional strategies to improve PCa outcomes, positively influence treatment selection and improve recommendations for patients with advanced PCa.

Currently, little is known about the impact of dietary tomato and its primary bioactive, lycopene, on PCa progression and the tumor microenvironment following treatment. We have previously demonstrated that tomato feeding reduced gene expression of cell cycle progression, proliferation, and androgen signaling markers in early carcinogenesis in TRAMP mice.[97] In rodent models of PCa, dietary lycopene has been shown to downregulate gene expression of autocrine and paracrine growth factors, reduce carcinogenesis, and inhibit tumorigenesis.[97-102] Lycopene and other tomato carotenoids accumulate in the prostate gland where they may protect DNA from oxidative damage and early carcinogenetic events.[103,104]

Lycopene has been shown to upregulate interleukin (IL)-12 and interferon (IFN)-γ in cultured cells.[101,105] IL-12 is a key negative regulator of angiogenesis by stimulating IFNγ, which mediates production of anti-angiogenic chemokines.[106] Other studies have found that lycopene may be anti-angiogenic by inhibiting matrix metalloproteinase 2 (MMP-2) and urokinase-type plasminogen activator (UPA) through vascular endothelial growth factor receptor (VEGFR)-2 mediated PI3K-Akt and ERK/p38 signaling pathways, leading to decreased vessel formation, invasion and metastasis.[101,107-109] Within these studies, there was also down-regulated expression of ras-related C3 botulinum toxin substrate (Rac)-1 and upregulated expression of tissue inhibitors of metalloproteinase-2 (TIMP-2) and plasminogen activator inhibitor-1 (PAI-1) which have been suggested to decrease cell migration and invasion.[107]

Current research and preliminary data suggest that feeding TP can reduce *de novo* androgen synthesis, which is critical for tumor growth and is the target of several PCa therapies such as ADT.[97,110] Lycopene, and other tomato bioactives may improve the effectiveness of radiation therapy and ADT by improving inflammatory status,[104] androgen and growth factor signaling,[97,110] apoptosis,[104,111,112] and cell cycle progression.[104,111,112] Recent evidence further supports that the whole food (tomatoes) is more effective than the individual bioactive (lycopene) as a potential adjuvant or chemopreventive agent.[103,113-115] Even though lycopene has therapeutic potential, our lab has shown using a chemically-induced model of PCa that TP, but not lycopene, resulted in longer survival compared to a control diet in our lab.[114] Additionally, our laboratory and others have revealed that tomato feeding reduces PCa incidence.[113,116,117] We have also shown that a 10% TP diet was effective at reducing tumor weight and cellular proliferation, while increasing apoptosis in the Dunning transplantable tumor model.[115] In addition to the anti-carcinogenic properties of tomatoes and lycopene, our previous data also suggest that there is great potential of tomatoes and lycopene to improve therapeutic outcomes that have not been explored.

Growth of primary PCa and CRPC tumors are driven by androgens. Tumors that relapse during ADT were originally believed to be “androgen independent”, but very low levels of androgens may still be detectable in tissue and serum of advanced patients.[36,37,118] Alternative pathways have been discovered whereby androgens can arise and accumulate within the tumor despite ADT.[36,37,118] Androgen receptors in patients with CRPC are expressed at similar levels to those in androgen-stimulated PCa and benign hyperplasia.[36,37,119] Additionally, androgen-related genes are frequently

expressed in CRPC tumors despite the reduction in signals from androgen deprivation therapy.[37,120] Alternative pathways have also been discovered by which androgens are still being expressed within the tumor.[36,37,118] The gene that codes for prostate specific antigen (PSA) is controlled by androgens acting on the androgen receptor.[121] Interestingly, tomato supplementation has been shown to reduce serum PSA levels.[117,122] This lowered concentration of PSA consistent with a decrease in androgen concentration within the prostate tumors.[121] Additionally, we have previously demonstrated that tomato or lycopene consumption reduces mRNA expression of enzymes that are important for de novo synthesis of androgens.[97] Consistent with our preliminary data and other studies, tomato or lycopene consumption appears to have a beneficial effect on the androgen axis by reducing testosterone and dihydrotestosterone biosynthesis (Figure 1.3). These results suggest that tomato and lycopene may exert protective effects for primary PCa and CRPC through modulation of the androgen axis.

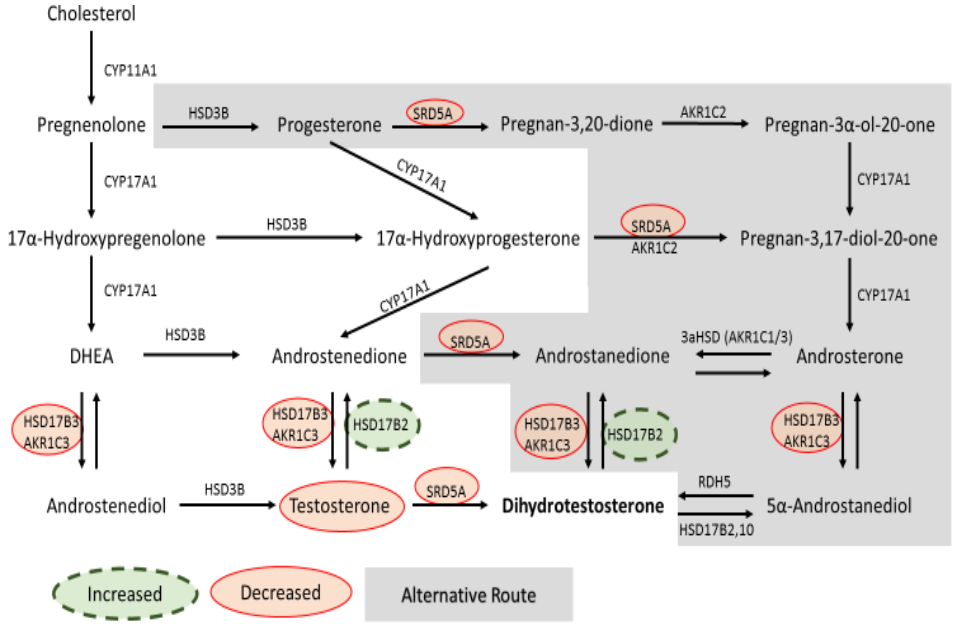


Figure 1.3. Proposed benefit of tomato and lycopene consumption on the androgen axis based on observed results in animal studies.

17β-hydroxysteroid dehydrogenase 3 (Hsd17b3) catalyzes the reduction of the inactive androstenedione to active testosterone and also acts on 5α-reduced androgens, converting androstanedione to the most potent androgen, dihydrotestosterone (DHT).[123] Conversely, 17β-hydroxysteroid dehydrogenase 2 (Hsd17b2) catalyzes the reverse reaction, oxidizing testosterone and DHT back to their inactive precursors (Figure 1.4). The balance between Hsd17b2 and Hsd17b3 activity may be critical, as *Hsd17b2* mRNA expression is low and *Hsd17b3* expression is high in malignant prostate tissue.[124] Thus, an elevated *Hsd17b2:Hsd17b3* ratio may contribute to net inactivation of intratumoral androgens and a less aggressive disease phenotype. Unpublished data from a previously mentioned TRAMP study demonstrated that <u>*tomato feeding increased the ratio of Hsd17b2:Hsd17b3 gene expression in primary tumors more than 2.5-fold*</u> (Figure 1.4).[116] Additionally, tumor and prostate tissue weight was reduced 42% in tomato-fed animals compared to control-fed mice (data not shown). These results suggest that tomato and lycopene may exert protective effects for primary PCa and CRPC through modulation of the androgen axis.

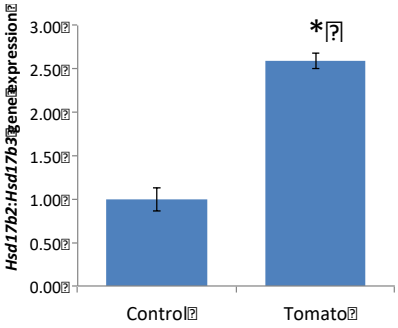


Figure 1.4**.** Gene expression ratio of *Hsd17b2:Hsd17b3* in tumors of 18-week-old TRAMP mice fed control or 10% tomato powder diets for 14 weeks unpublished data (n= 6 per diet).

Levels of evidence

Based on the epidemiological and preclinical evidence for PCa, many men with PCa might choose to consume lycopene supplements without evidence from definitive phase III human trials. One of the primary goals of evidence-based research is to provide a scientific foundation to inform clinical decisions. A cornerstone of evidence-based

research is a hierarchical classification system known as the levels of evidence.[125] Clinicians and patients are encouraged to utilize information from the highest level of evidence possible to encourage their decisions.

Systematic reviews of randomized control trials (RCTs) produce the highest level of evidence for therapeutic studies and are the most reliable source of determining causal relationships between dietary exposures and prostate carcinogenesis. Currently, the highest level of evidence for tomatoes and PCa are found in cohort studies, which are not capable of determining causal relationships. Importantly, most cohort studies did not report sources of tomatoes that were thermally processed or consumed with dietary fat.[126] The exclusion of these tomato products results in reporting of foods with low absorption of lycopene (such as seen with raw tomatoes)[53-55] and other phytochemicals that might modify the growth and progression of a prostate tumor. Systematic reviews and dose-response meta-analyses are needed to increase the reliability of the hypothesis that tomatoes and lycopene are associated with decreased prostate carcinogenesis.

Although reducing prostate carcinogenesis is preferable to treating PCa, studies have not evaluated the role of tomato feeding within therapeutic approaches for PCa. Following PCa diagnosis, patients often seek information about food and supplements that may improve their response to therapies, quality of life, and survival. Data regarding the interactions between tomato consumption and therapeutic approaches are critically needed because it is not currently known if tomatoes will improve or worsen the intended treatment. Lycopene and other carotenoids that are present within tomatoes are potent antioxidants that may improve natural defenses by scavenging free radicals generated during radiolysis. This property of lycopene would reduce damage to surrounding tissues,

but this same property could also reduce the effectiveness of the radiation therapy within the prostate tumor. More data are required to determine whether tomato products influence the efficacy of prostate cancer treatment.

Aims of this Dissertation

Following prostate cancer (PCa) diagnosis, men often seek information about food and supplements that may improve their response to therapies, quality of life and survival. If PCa is detected early, common therapeutic options include: active surveillance, surgery (prostatectomy), radiation therapy and/or chemotherapy.[11,12] Despite primary treatment, approximately 30% of men suffer tumor recurrence.[31] These men, along with men diagnosed with advanced or metastatic cancer, usually undergo ADT.[31,32] While current therapies are initially successful for primary PCa, combinatorial dietary techniques are increasingly needed to protect against PCa progression and to alleviate adverse effects associated with from common therapies.

The proposed studies include two dose response meta-analyses of the published literature associated with tomato or lycopene exposure and PCa risk. The proposed studies also include two preclinical studies utilizing the TRAMP mouse model to provide critical data on the ability of tomato or lycopene consumption to enhance clinical outcomes of PCa therapies (EBRT and ADT) that may reduce progression, improve quality of life, and increase survival. Elucidating whether these dietary approaches can improve therapeutic outcomes should positively affect treatment selection and recommendations for patients. Therefore, the aims of this dissertation are the following:

1. Evaluate the epidemiological associations between the exposures of tomatoes or lycopene and risk of prostate cancer in the epidemiological literature.

Hypothesis 1: We hypothesized that increased tomato consumption would be associated with a reduced risk of PCa incidence.

Hypothesis 2: We hypothesized that increased lycopene exposure (dietary and blood) would be associated with a reduced risk of PCa incidence.

2. Determine the potential of tomato or lycopene interventions to reduce the growth and progression of CRPC in the TRAMP model

   Hypothesis: We hypothesized that tomato and lycopene interventions would reduce the incidence of CRPC tumors and would reduce the growth of CRPC tumors over 5 weeks of ultrasound monitoring.

3. Evaluate the potential for lyophilized tomato powder to reduce radiation-induced apoptosis and cell death in prostate tumors and surrounding tissues in the TRAMP model.

   Hypothesis 1: We hypothesized that dietary lyophilized tomato powder (TP) would not reduce apoptosis or cell death scores within the prostate tumor following external beam radiation therapy (EBRT).

   Hypothesis 2: We hypothesized that TP would reduce apoptosis and cell death scores within the tissues surrounding the prostate tumor following EBRT.

Chapter 2: Processed and raw tomato consumption and risk of prostate cancer: a systematic review and dose–response meta-analysis

[a]*Abstract*

Background: Prostate cancer (PCa) is the second most frequently diagnosed cancer among men worldwide. Many epidemiological studies have found an inverse association between increased tomato consumption and PCa risk. This study aims to determine the associations between consumption of various types of tomato products and PCa risk and to investigate potential dose-response relationships.

Methods: We conducted a systematic review and dose-response meta-analysis of dietary tomato in relation to PCa. Eligible studies were published before April 10, 2017 and were identified from PubMed, Web of Science, and the Cochrane Library. We estimated pooled risk ratios (RR) and 95% confidence intervals (CI) using random and fixed effects models. Linear and nonlinear dose-response relationships were also evaluated for PCa risk.

Results: Thirty studies related to tomato consumption and PCa risk were included in the meta-analysis, which summarized data from 24,222 cases and 260,461 participants. Higher total tomato consumption was associated with a reduced risk of PCa (RR=0.81, 95% CI: 0.71-0.92, p=0.001). Specifically, tomato foods (RR=0.84, 95% CI: 0.72-0.98, p=0.030) and cooked tomatoes and sauces (RR=0.84, 95% CI: 0.73-0.98, p=0.029) were associated with a reduced risk of PCa. However, no associations were found for raw

[a] The content of this chapter has been published (Reference 127. Rowles JL, Ranard KM, Applegate CC, Jeon S, An R, Erdman JW. Processed and raw tomato consumption and risk of prostate cancer: a systematic review and dose–response meta-analysis. *Prostate Cancer and Prostatic Diseases.* 2018;21(3):319-336.).

tomatoes (RR=0.96, 95% CI: 0.84-1.09, p=0.487). There was a dose-response association observed for total tomato consumption (p=0.040), cooked tomatoes and sauces (p<0.001), and raw tomatoes (p=0.037), but there was not a significant association with tomato foods (p_{linear}=0.511, $p_{nonlinear}$=0.289).

Conclusions: Our data demonstrates that increased tomato consumption is inversely associated with PCa risk. These findings were accompanied with dose-response relationships for total tomato consumption and for cooked tomatoes and sauces. Further studies are required to determine the underlying mechanisms of these associations.

Introduction

Prostate cancer is the second most common cancer in men and is the fifth leading cause of cancer-related deaths worldwide.[1] The development of PCa is complex and is influenced by a combination of genetic, hormonal and environmental factors.[97] Diet is one of the most modifiable risk factors for PCa. Many epidemiological studies have investigated tomato products and their association with PCa carcinogenesis. Despite much positive data, studies have yielded mixed results. Lycopene, a lipophilic carotenoid that gives some fruits and vegetables their red color, is believed to be the primary bioactive component in tomatoes.[44,45] Meta-analyses of observational studies indicate that increased lycopene exposure (diet or circulating blood concentrations) is associated with a reduced risk of PCa.[128-132] In the United States, more than 85% of dietary lycopene comes from tomato products, which suggests that lycopene could be a surrogate biomarker of tomato consumption.[44,45,133] In addition to lycopene, tomatoes contain other carotenoids (i.e., phytoene and phytofluene) and polyphenols that accumulate in the prostate and may elicit protective anticarcinogenic effects.[134-136]

Three previous meta-analyses have investigated the association between tomato consumption and PCa risk.[128,130,137] Among these analyses, none found an association with raw tomatoes,[128,130] one found an association with cooked tomatoes,[130] and one found an association by mixing risk estimates of multiple tomato products together.[137] However, previous analyses have not investigated the association between tomato consumption and advanced PCa risk. Additionally, none of the previous analyses investigated potential dose-response associations. The current study systematically and quantitatively evaluates the associations between dietary tomato and PCa risk. This meta-analysis enhances the existing literature about dietary tomato and its associations with PCa by investigating multiple forms of tomatoes (including tomato foods, cooked tomatoes, sauces, raw tomatoes and pizza), which provides a more comprehensive view of the associations between tomato consumption and PCa risk. This is also the first meta-analysis that evaluates the relationship between tomatoes and advanced PCa. It is also the first to investigate trends over time through cumulative meta-analyses and potential dose-response associations.

Materials and Methods

Study selection criteria

This meta-analysis was conducted in accordance with the PRISMA guidelines and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines.[138,139] Studies that met the following criteria were included in this meta-analysis: (a) evaluated the association between tomato products and PCa risk by using randomized control trials, prospective, retrospective, cross-sectional, and case control studies; (b) methodology

was documented in replicable detail; (c) included relative risk ratio with 95% confidence intervals (CIs) for exposure categories; (d) articles were written in English; and (e) peer-reviewed publications or theses. Additionally, studies that provided doses of tomato products, length of intervention, and relative risk for PCa were included in the dose-response meta-analysis.

Literature search

We conducted a comprehensive literature search in PubMed, Web of Science and the Cochrane Library from their inception to April 10th, 2017 using the following key words: prostate cancer, prostate neoplasm, tomato, carotenoids, humans, case-control studies, follow-up studies, cohort, prospective studies and their variants. Titles and abstracts of articles that were identified by the keyword search were screened against the study selection criteria. Potentially relevant articles were independently retrieved for evaluation of the full text by two authors (JR3 and CA). We also conducted a reference list search (i.e., backward search) and cited reference search (i.e., forward search) from full text articles meeting the study selection criteria. Articles identified through this process were further screened and evaluated using the same criteria. We repeated searches on all newly identified articles until no further relevant articles were found. Two authors (JR3 and KR) individually determined inclusion/exclusion of all articles retrieved in full text and discrepancies were resolved through discussion.

Data extraction and quality assessment

The following information was extracted from each study: name of first author; year of publication; location of study; study period; number of cases, controls, total number of participants in the study; age of participants; years until follow-up; exposure values of

tomato sources consumed (raw tomato, cooked tomato, tomato sauces, multiple/undescribed sources, and pizza); relative risk ratios for prostate cancer; adjustments for covariates; and study type. Data extraction from each study was independently conducted by two authors (JR3 and SJ). Authors were contacted for additional data for studies that had missing values for tomato exposure, relative risk for PCa incidence, or the number of cases. The term RR (relative risk) will be used as a generic term for relative odds (cumulative incidence data), rate ratio (incidence-rate data), odds ratios (case-control data) and for hazard ratios (HR). The quality of each study was evaluated by two authors (JR3 and KR) using the Newcastle-Ottawa Scale, which is a validated scale for non-randomized cohorts in a meta-analysis.[140] This tool judges the literature based on three broad categories: selection of cases and controls; comparability of studies; and exposure of the main variable. We regarded scores of 1-3, 4-6 and 7-9 as low, medium and high quality, respectively. The quality score helped to measure the strength of each study's evidence and was not used to determine the inclusion of studies. Other aspects of study quality were analyzed in the subgroup analysis.

Statistical analysis

RRs and 95% CIs were used as a measure of the effect size for all studies because HR and odds ratios (OR) are approximately equal to RR for low incidence of diseases.[141] Heterogeneity among studies was explored by using Cochran's test and I^2 statistic.[142] Fixed and random (DerSimonian-Laird) effects were applied based upon the I^2 value as a marker of study heterogeneity.[142] If there was low to moderate heterogeneity between studies (I^2<50%) a fixed effect model was used to determine RR estimates. If heterogeneity between studies was moderate to substantial (I^2>50%), a random effects

model was applied. When results from fixed and random effects models were different with moderate heterogeneity (I^2: 30-60%), we presented the latter as it represents a more conservative approach.[143,144]

If tomato consumption was reported by the number of servings per unit time, we followed the U.S. Food and Drug Administration's (FDA) Code of Federal Regulations (CFR) Title 21 Part 101 regulation for food labeling (http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=101) to approximate the number of grams per serving. The number of grams per serving were as follows: 148 grams per serving for raw tomato; 60 grams per serving for tomato sauces or cooked tomatoes; 104 grams per serving (1:1 ratio of raw and cooked tomato products) for tomato foods. Tomato foods were defined as exposure categories that did not indicate the source of tomato or combined multiple exposure categories (i.e., cooked and raw tomatoes). Details on the exact items included for each study can be found in Table B.1. We utilized the following hierarchy of lycopene bioavailability from tomato sources based on previous literature: tomato sauce = cooked tomatoes > tomato foods > raw tomatoes > pizza.[53,54,57,58] Pizza was considered the lowest bioavailable group due to the heterogeneity of tomato sauce (if present) and differential preparation methods.

The study-specific RR for the highest quantile was compared to the lowest quantile of each dietary tomatoes source because studies report different exposure categories as tertiles, quartiles and quintiles. Potential publication bias was assessed by using funnel plots[145,146] with Egger's linear regression test[147] and Begg's rank correlation test of asymmetry.[148] If evidence of asymmetry was indicated, the trim-and-fill method was used to recalculate adjusted estimates with the addition of missing studies.[149] We also

performed sensitivity analyses to evaluate whether the pooled results could have been affected by excluding a single study at a time. Subgroup analyses were performed on study type, location of study and covariate adjustment. If the total number of cases or person-years were presented without distribution, we estimated the distribution by dividing by the number definitions of the quantiles.

For the meta-analysis of the dose-response association between tomato consumption and PCa risk, the method of generalized least squares for trend estimation proposed by Greenland and Longnecker was utilized.[150,151] Based on data for each level of tomato consumption, study specific slopes (with 95% CIs) were generated. Additionally, we examined linear and nonlinear associations between tomato consumption and PCa risk by plotting linear and nonlinear dose-response curves using restricted cubic spines, with 3 knots at 10%, 50% and 90% of the distribution. A p-value for curve linearity and nonlinearity was calculated by testing that the coefficient of the second spline was equal to zero. All data were analyzed by STATA/IC version 14.2 (StataCorp LP, College Station, TX). A value of $p<0.05$ was considered significant for all statistical analyses.

Results

Literature search

The initial abstract screening and reference list search yielded a total of 1,825 articles after removing duplicates. After screening these titles and abstracts, 74 articles remained for full-text evaluation. Among the 44 excluded articles, 21 did not discuss tomato consumption,[152-172] 19 did not indicate risk for prostate cancer,[122,173-190] 2 included

data that were included in other studies/later follow-ups,[191,192] and 2 had an irrelevant topic[193,194]. Among these articles, 30 were included and 44 were excluded. From the initial 30 articles that were included in the meta-analysis, 19 studies investigated the association of tomato foods,[179,195-212] 10 investigated raw tomatoes,[195,201,205,208,213-218] 7 investigated tomato sauce,[201,208,217,219-222] 6 investigated cooked tomatoes,[202,208,213,215,216,218] and 4 investigated pizza[201,208,217,223] (Figure 2.1).

Study characteristics

Table 2.1 summarizes the 30 studies that investigated the association between tomato consumption on PCa risk. Among these studies, 21 were case-control studies[179,195,197-200,202,203,205-207,209,211,212,214-216,218,221-223] and 9 were cohort studies [196,201,204,208,210,213,217,219,220]. These studies had a combined total of 24,222 cases and 260,461 participants. Twelve studies were from North America,[179,195,199,201,202,204,208,211,216,217,219,220] 8 were from Europe,[196,197,205,212,214,215,218,223] 8 were from Asia,[200,203,206,207,209,210,221,222] and 2 were from Australia.[198,213] All studies that reported intake values utilized some form of a food frequency questionnaire. Many of the studies provided risk estimates that were adjusted for age (n=26),[179,195-208,210,211,213-217,219,220,222,223] energy (n=19),[179,195-202,206,207,212,215-217,219,220,222,223] family history of prostate cancer (FHPC, n=17),[179,195-198,200,201,206,208,211,214,216,217,219,220,222,223] body mass index (BMI, n=16),[195,196,199-201,203,206,208,210,211,214-217,219,220] smoking (n=13),[196,197,200,201,203,206,207,210,211,217,219,220,223] physical activity (n=7),[196,201,214,217,219,220,223] alcohol intake (n=8),[196,203,206,210,217,219,220,223] or education (n=8).[195,196,199,200,202-204,208]

The Newcastle-Ottawa Scale was utilized to assess quality of all included studies. As described in Table 2.2, the highest quality score was 8 and the lowest was 4. The

average quality score was 6.24 (SD: 1.14) for case-control studies and 7.14 (SD: 1.46) for cohort and nested case-control studies. As a result, cohort studies were considered higher quality and case-control were considered medium quality.

Tomato consumption and PCa risk

All of these studies had complete relative risk information and were included in the meta-analysis. Because several studies reported risk associations from more than one type of tomato food (i.e., raw or cooked tomatoes), we analyzed the RR from the type of tomato with the highest lycopene bioavailability. Figure 2.2 displays the pooled RR of PCa when comparing the highest tomato consumption category to the lowest. The pooled RR for this association was 0.81 (95% CI: 0.71-0.92, p=0.001, I^2=73.1%). Specifically, the pooled RR was 0.68 (95% CI: 0.55-0.84, p<0.001, I^2=77.4%) and 0.92 (0.86-0.98, p=0.013, I^2=41.1%) for case-control and cohort studies, respectively (Figure B.1). Furthermore, we investigated publication bias by funnel plot (Figure B.2A), Begg's correlation test and Egger's linear regression test. Begg's correlation test (p=0.003) and Egger's linear regression test (p=0.011) for bias were significant.

We further stratified studies by the type of tomato consumption and evaluated associations with PCa risk. Studies that investigated tomato foods (RR=0.84, 95% CI: 0.72-0.98, p=0.030, I^2=76.7% [Figure 2.3]) or cooked tomatoes and sauces (RR=0.84, 95% CI: 0.73-0.98, p=0.029, I^2=57.4% [Figure 2.4]) were inversely associated with PCa risk. However, raw tomatoes (RR=0.96, 95% CI: 0.84-1.09, p=0.487 [Figure 2.5], I^2=55.6%) and pizza consumption (RR=1.02, 95% CI: 0.85-1.22, p=0.850 [Figure 2.6]) were not associated with PCa risk.

Subgroup analysis of tomato consumption and PCa risk

Several subgroup and sensitivity analyses were conducted for total consumption of tomatoes. A statistically significant result was first achieved in 1999 and has remained unchanged after 22 additional studies were published (Figure B.3). There were no studies that strongly influenced the heterogeneity of the dataset. Table 2.3 describes the subgroup analyses for associations between tomato consumption and PCa risk. Studies conducted in North America were not significant (p=0.501), however studies conducted in Europe (p=0.029) and other continents (p=0.001) were significant. Interestingly, studies that did not adjust for BMI (p=0.002), smoking (p=0.002), FHPC (p=0.003), education (p=0.001), physical activity (p<0.001) and alcohol (p=0.001) were associated with a decreased risk of PCa.

For tomato foods, the pooled RR for case-control studies was 0.69 (95% CI: 0.53-0.91, p=0.008, I^2=80.3%) and 1.00 (95% CI: 0.87-1.15, p=0.963, I^2=57%) for cohort and nested case-control studies. Publication bias was explored with a funnel plot (Figure B.2B). Begg's correlation test (p=0.053) was not significant while Egger's linear regression test (p=0.037) for bias was significant. No specific studies strongly affected the pooled RR, heterogeneity or publication bias. In the cumulative meta-analysis, the pooled RR fluctuated with time and remained significant after 2011 (Figure B.3). Studies conducted in North America (p=0.800) and Europe (p=0.424) were not significant, while studies conducted in other continents (p=0.010) were significant. Studies that did not adjust their model for age (p=0.005), BMI (p=0.006), FHPC (p=0.026), education (p=0.025), physical activity (p=0.010), or alcohol (p=0.032) were significantly associated with a reduced risk of PCa.

Regarding cooked tomatoes and sauces, the pooled RR was 0.63 (95% CI: 0.40-1.00, p=0.052, I^2=69.1%) for case-control studies and 0.92 (95% CI: 0.85-0.99, p=0.025, I^2=0%) for cohort and nested case-control studies. Publication bias was explored by funnel plot (Figure B.2C). Begg's correlation test (p=0.020) and Egger's linear regression test (p=0.019) for bias were significant. No specific studies strongly impacted the pooled RR, heterogeneity or publication bias. Studies conducted in North America were significantly associated with PCa risk (p=0.033), however studies conducted in Europe (p=0.202) and other continents were not (p=0.064). Additionally, no significant trends were found in the cumulative meta-analysis (Figure B.4). Studies that adjusted for age (p=0.006), BMI (p=0.012), FHPC (p=0.028), and energy (p=0.008) were significantly associated with a reduced risk of PCa.

For raw tomatoes, the pooled RR was 0.95 (95% CI: 0.76-1.19, p=0.729, I^2=55.9%) for case-control studies and 0.96 (95% CI: 0.81-1.14, p=0.557, I^2=60.6%) for cohort and nested case-control studies. Publication bias was explored with a funnel plot (Figure B.2D). Begg's correlation test (p=0.592) and Egger's linear regression test (p=0.568) for bias were both not significant. The pooled RR, heterogeneity and publication bias were not strongly affected by any specific studies. Although the pooled RR for raw tomato consumption was initially significant in 1995, this association has remained not significant since 2000 after the addition of 7 studies (Figure B.5). Studies conducted in North America (p=0.804) and other continents (p=0.889) were not associated with PCa risk, however studies conducted in Europe were significantly associated with a reduced risk of PCa (p=0.008). No specific adjustments were associated with a reduced risk of PCa other than alcohol (p=0.012).

For pizza consumption, the pooled RR was 1.12 (95% CI: 0.88-1.43) for one case-control study and 0.97 (95% CI: 0.74-1.26, p=0.818, I^2=72%) for cohort studies. Publication bias was explored with a funnel plot (Figure B.1E). Begg's correlation test (p=0.734) and Egger's linear regression test (p=0.568) for bias were both not significant. In the cumulative meta-analysis, pizza consumption was not significant in the first study and has remained not significant after 3 additional studies were published (Figure B.6). Due to the low number of studies that reported risk associations for pizza, subgroup and sensitivity analyses were not pursued.

Tomato consumption and advanced PCa risk

Four studies investigated the association between tomatoes and advanced PCa.[179,202,208,219] Of these studies, one classified advanced PCa by stage,[219] one by grade,[208] and two used both stage and grade.[179,202] Additionally, all studies that investigated associations between tomato consumption and advanced PCa were high quality. We explored publication bias with a funnel plot (Figure B.2F). Begg's correlation test (p=0.089) and Egger's linear regression test (p=0.112) for bias were both not significant. The pooled RR for this association was 0.89 (95% CI: 0.77-1.03, p=0.113, I^2=35.3% [Figure 2.7]). Specifically, the pooled RR was 1.10 (95% CI: 0.84-1.44, p=0.493, I^2=0%) for case-control studies and 0.81 (95% CI: 0.68-0.97, p=0.019, I^2=16.3%) for cohort studies. Due to the low number of studies that reported risk associations for advanced PCa, subgroup and sensitivity analyses were not pursued.

Dose response

Of the studies that were eligible for dose-response analysis, 19 investigated tomatoes;[195,196,198-207,210,213-216,218,220]13 investigated tomato foods;[195,196,198-207,210] 9

investigated raw tomatoes;[195,201,205,213-218] and 7 investigated cooked tomatoes and sauces.[201,202,213,215,216,218,220] Every study included in the dose-response contained relevant risk estimates with information for the dose of each quantile reported. Due to a lack of dose data, we did not conduct a dose-response meta-analysis on associations between pizza consumption and PCa and between tomato consumption and advanced PCa. Dose-response associations are described in Figure 2.8. Dose responses according to the type of study are described in Figure B.7.

First, we investigated the dose-response association between total tomato consumption and PCa risk. One study that substantially increased the heterogeneity of the data was excluded from the dose response estimate (Figure B.8A).[206] After this study was excluded, the error in the estimate was substantially decreased, and there was a significant nonlinear dose-response association between tomato consumption and PCa. As seen in Figure 2.8A, there was a significant nonlinear dose-response association between tomato consumption and PCa risk (p_{linear}=0.099, $p_{nonlinear}$=0.017). PCa risk decreased by 13% at 200 grams/week, 28% at 500 grams/week, 46% at 1,000 grams/week, and 56% for 1350 grams/week.

We investigated, but did not find any dose-response associations between consumption of tomato foods and risk of PCa (Figure B.8B, p_{linear}=0.400, $p_{nonlinear}$=0.173). In the sensitivity analyses, one study substantially increased the heterogeneity of the data[206]. After this study was excluded, the error in the estimate was substantially decreased, but the dose-response meta-analysis remained nonsignificant (Figure 2.8B, p_{linear}=0.448, $p_{nonlinear}$=0.276).

We investigated the dose-response associations between cooked tomatoes and sauces in relation to risk for PCa. We identified a significant dose-response association between cooked tomatoes and sauces and PCa risk (Figure 2.8C, $p_{linear}<0.001$, $p_{nonlinear}<0.001$). PCa risk decreased by 3% for 60 grams/week, 12% for 120 grams/week, 19% for 240 grams/week, and 49% for 420 grams/week in the nonlinear model and decreased by 3.5% for each additional 30 grams/week. No studies significantly affected the heterogeneity of the data.

Lastly, we investigated the dose-response associations between raw tomato consumption and risk of PCa. A significant linear dose-response association was observed between raw tomatoes and risk of PCa (Figure 2.8D, $p_{linear}=0.037$, $p_{nonlinear}=0.099$) such that PCa risk decreased by 2% for each additional 100 grams of raw tomatoes consumed per week. No studies significantly affected the heterogeneity of the data.

Discussion

The present systematic review and meta-analysis analyzed 30 studies, which included 24,222 cases with PCa reported from 260,461 participants. This analysis demonstrated inverse associations between PCa risk and consumption of tomatoes (total); tomato foods; and cooked tomatoes and sauces. No associations were found for raw tomatoes or pizza and PCa risk, nor were associations found between tomato consumption and advanced PCa risk. Due to a low number of studies investigating advanced PCa, associations may have been missed. These associations were further supported by dose-response associations for total tomato consumption and for cooked

tomatoes and sauces. To our knowledge, this is the first meta-analysis to investigate potential dose-response associations of tomato consumption. Our data demonstrate that increased tomato consumption (particularly cooked tomatoes and sauces) could reduce PCa incidence.

In a recently published pooled analysis, total tomato consumption was not associated with a reduced risk of PCa.[126] Petimar, et al. elaborated that the vast majority of prospective cohort studies did not assess sources of bioavailable lycopene (i.e., cooked tomatoes and sauces).[126] Associations in their study may have been missed if lycopene was not able to be adequately absorbed or metabolized. Generally, increased lipid consumption (particularly with long chain fatty acids) improves lycopene and carotenoid bioavailability by increasing solubility, and thermal food processing improves carotenoid bioavailability by disrupting cellular membranes, which allows lycopene to be released from the tissue matrix.[53,54,56-58] Our review and others[128,130] have failed to find an association between raw tomatoes and PCa risk. However our review and one other have found an inverse association between PCa risk and cooked tomatoes and sauces.[130] We were unable to account for different preparation methods and the volume of tomato sauce (if present) on the pizza. As a result, associations with pizza may have been missed. Overall, these results further support the hypothesis that bioavailable lycopene and other tomato carotenoids are associated with a reduced risk of PCa.

Tomatoes contribute up to 85% of dietary lycopene in the United States.[44,45] In our recent meta-analysis of lycopene and PCa risk, lycopene consumption was associated with a 12% reduced risk of PCa and an estimated 1% reduced risk for each additional 14 mg consumed per week.[132] The current study suggests a more robust reduction of PCa

risk than our recent dose-response estimates for lycopene.[132] From the current study, there was an estimated 9% reduced risk for 100 grams/week of cooked tomato. For an equivalent dose of lycopene (22 mg lycopene in 100 grams of tomato puree according to the USDA Nutrient Database), tomatoes were more effective than lycopene at reducing PCa risk (9% compared to 1.6%). The greater benefit from tomato products may be due to interactions between potentially beneficial bioactive compounds in tomatoes.[44,45,134] Additional research is needed to understand the benefit of tomato bioactives other than lycopene that may contribute to the reduced risk of PCa.

The present analysis is the first to investigate the associations between tomato consumption and advanced PCa risk. Although increased tomato consumption was associated with a reduced risk of PCa, no association was observed for tomatoes and advanced PCa. Many studies did not include the grade or stage of PCa and contained only a small number of cases with advanced stages of PCa. Due to the small number of studies that investigated advanced PCa, an association may have been missed. A recent pooled analysis of prospective cohort studies found that pizza with tomato sauce was associated with a reduced risk of lethal PCa.[126] As previously mentioned, lycopene is also a biomarker for tomato consumption.[44,45,133] Another pooled analysis of prospective cohort studies found that increased circulating lycopene was associated with a reduced risk of advanced PCa.[94] Combined, these pooled analyses suggest that tomato consumption may be associated with advanced PCa. A better understanding of the associations between the stage or grade of PCa and carotenoid status may lead to a reduced progression of PCa. More studies are needed in order to elucidate the associations and potential benefits of tomato consumption on advanced PCa risk.

Our analysis exhibits several strengths that contribute to the literature. Dose-response meta-analyses along with meta-analyses for comparisons of high to low tomato consumption provides additional data points that were not considered in previous reviews. Our additions further strengthened the associations found in this study and those of other reviews. Cumulative meta-analyses assisted in evaluating trends over time and improved the interpretation of the data. Sensitivity and subgroup meta-analyses were conducted to examine the sources of heterogeneity and evaluate associations between tomato consumption and PCa risk.

Despite these strengths, this study has several limitations that should be noted. First, the specific types of tomatoes consumed and the preparation methods varied in each study. A higher composition of raw tomatoes rather than cooked tomatoes may have reduced the bioavailability of lycopene and other carotenoids and may have reduced the association that was found. We were also somewhat limited in our ability to detect dose-response associations because tomato consumption was inconsistently reported across studies. Some studies reported servings of tomatoes per week, while others reported grams per week. Next, guidelines for PCa screening varies between countries, medical organizations and guideline groups and may affect PCa detection and incidence rates.[5,6] Screening for PCa is an important factor of PCa diagnosis, especially of non-aggressive disease.[3] Along these lines, stratified analyses of pre-PSA and post-PSA era in the United States and Europe may be useful. To this point, a recent pooled analysis of circulating lycopene found an inverse association with a reduced risk of PCa before the PSA era (1990).[94] Unfortunately, only one study was conducted with a study period that ended before 1990, so we were limited in this probing question. In the current study, cohort

studies had relatively modest inverse associations with PCa risk compared to case-control studies. It is important to note that a single dietary assessment will have significant measurement error for both types of studies. To improve the estimate of the associations for tomato consumption and to further explore the associations of the different forms of tomato consumption, both types of studies were included.

In conclusion, our data indicate an inverse association between tomato consumption and PCa risk. This study further supports the protective role of tomatoes and lycopene in prostate carcinogenesis. These data were further supported with dose-response associations between total tomato consumption and cooked tomatoes and sauces and PCa risk. Our results, along with those of other meta-analyses, suggest potential health benefits from increasing tomato consumption. Promoting tomato consumption to achieve protective levels observed in this analysis is relatively easy. For example, our results suggest that 245 grams of tomato sauce per week would approximately provide a 30% reduced risk of PC. According to the USDA National Nutrient Database, 1 cup of tomato sauce weighs 245 grams. Our results support increasing tomato consumption in order to reduce PCa incidence. More high-quality research is required in order to elucidate the underlying mechanisms behind this inverse association.

Tables and Figures for Chapter 2

Table 2.1: Characteristics of included studies.

Source Author, Year	Study Period	Country	Number of cases	Number of controls	Total no. participants in cohort	Age (SD)	Study Type	Tomato exposure and dose (grams/week)	Adjustments
Ambrosini, 2008	1994-2004	Australia	97	1,888	6,493	Cases: 62.6 Controls: 54.7	Nested Case-Control	Raw tomatoes T1<251.6, T3>606.8 Cooked tomatoes T1<60, T3>180	Age, vegetable and fruit intake, retinol and β-carotene supplementation, crocidolite exposure
Bosetti, 2000	1994-1997	Greece	320	246	566	Cases: 71.1 (8.0) Controls: 70.4 (7.9)	Case-Control	Raw tomatoes†, Cooked tomatoes†	Age, height, BMI, Edu, energy
Bosetti, 2004	1991-2002	Italy	1,294	1,451	2,745	Cases: 63 (46-74) Controls: 66 (46-74)	Case-Control	Raw tomatoes: T1<150.8, T3>345.8	Age, study center, Edu, occupational PA at 30-39, BMI, FHPC
Cohen, 2000	1993-1996	USA, Seattle	628	602	1,230	40-64	Case-Control	Raw tomatoes: T1<130, T3≥390 Cooked tomatoes: T1<148, T3≥444	Fat, energy, race, age, FHPC, BMI, PSA tests in last 5 yrs and Edu
Darlington, 2007	1995-1998	Canada	752	1,613	2,365	50-84	Case-Control	Raw tomatoes: Q1<130, Q4>390 Tomato foods: Q1<208, Q4>780	Energy, age, FHPC, BMI, Edu, occupation
Diallo, 2016	1994-2007	France	139	3,313	13,017	Cases: 63	Cohort	Tomato foods: T1<1,207.5; T3>1356.6	Age, energy, trt group, number of diet records, SS, Edu, PA, height, PA, height, BMI, PSA, alcohol, FHPC, calcium intake, dairy, plasma α-tocopherol and selenium
Er, 2014	2001-2009	United Kingdom	1,806	12,005	~110,000	Cases: 62.0 (5.0) Controls: 61.6 (5.0)	Nested Case Control, RCT	Tomato foods: Low ≤1040, high>1040	Age, recruitment center, FHPC, SS, energy

Table 2.1: Characteristics of included studies (continued).

Source Author, Year	Study Period	Country	Number of cases	Number of controls	Total no. participants in cohort	Age (SD)	Study Type	Tomato exposure and dose (grams/week)	Adjustments
Gallus, 2006	1991-2002	Italy	1,294	1,451	2,745	Cases: 63 (46-74) Controls: 66 (46-74)	Case-Control	Pizza: T1 ≤0.25, T3 ≥ 1 serving	Age, study center, Edu, BMI, SS, alcohol, energy, PA
Giovannucci, 1995 (HPFS)	1986-1992	USA	812	—	47,894	40-75	Cohort	Raw tomatoes Q1<0.25, Q4≥296 Tomato sauce: Q1<15, Q4≥120 Pizza: Q1<0.25 Q4≥2 servings	Age, BMI, aspirin, marital status, ancestry, location, PA, vasectomy, SS, intake of nutrients including alcohol
Giovannucci, 2007 (HPFS)	1986-2002	USA	3,029	—	51,525	40-75	Cohort	Tomato sauce: Q1<15, Q4≥120	Age, BMI, SS, height, PA, FHPC, diabetes, energy, nutrients, and Vit E
Graff, 2016 (HPFS)	1986-2005	USA	5,543	—	46,719	40-75	Cohort	Tomato sauce: Q1<15, Q4≥120	Age, time period, race, height, BMI, PA, SS, diabetes, FHPC, PSA, MV, energy, nutrients, alcohol, coffee, ERG
Hardin, 2011	2001-2004	USA, Ohio	470	512	982	Cases: 65.8 (8.3) Controls: 65.9 (8.5)	Case-Control	Tomato foods: Q1: 187.2, Q4: 1,019.2	Age, race, institution, energy, FHPC
Hodge, 2004	1994-1997	Australia	858	905	1,763	<70	Case-Control	Tomato foods: T1<364, T3 ≥676	Age, state, country of birth, SES, energy FHPC
Jain, 1999	1989-1993	Canada	617	636	1,253	Cases: 69.8 Controls: 69.9	Case-Control	Tomato foods: Q1<65.1, Q4≥767.2	Age, energy, vas, SS, marital status, study area, BMI, Edu, MV, nutrients
Jian, 2005	2001-2002	China	130	274	404	Cases: 72.7 (7.1) Controls: 71.4 (7.2)	Case-Control	Tomato foods: Q1<38.4, Q4≥235.3	Age, location, Edu, SES, income, BMI, marital status, tea consumption, FHPC, children, energy

Table 2.1: Characteristics of included studies (continued).

Source Author, Year	Study Period	Country	Number of cases	Number of controls	Total no. participants in cohort	Age (SD)	Study Type	Tomato exposure and dose (grams/week)	Adjustments
Key, 1997	1989-1992	United Kingdom	328	328	656	Cases: 68.1 Controls: 68.1	Case-Control	Raw tomatoes: Q1 ≤ 148, Q4 ≥ 740 Cooked tomatoes: Q1≤ 15, Q4≥240	Social class
Kirsh, 2006	1993-2001	USA	1,338	—	29,361	55-74	Cohort	Raw tomatoes: Q1<92.5, Q5>444 Tomato foods: Q1<385.8, Q5>1070.2 Tomato sauce: Q1<15, Q4>60 Pizza: Q1<0.50 Q4≥1 servings	Age, energy, race, study center, FHPC, BMI, SS, PA, Vit E, intake, diabetes, aspirin, PSA screens
Kolonel, 2000	1987-1991	USA and Canada	1,619	1,618	3,237	Cases: Up to 84	Case-Control	Tomato foods: Q1 ≤ 20.0 Q5 ≥ 108.1, Cooked tomatoes: Q1 ≤ 18.3 Q5 ≥ 92.7	Age, Edu, ethnicity, location, energy
Li, 2008	1998-2000	China	28	280	308	Cases: 71.4 (6.0) Controls 71.1 (5.8)	Case-Control	Tomatoes foods: T1 < 104, T3≥312	Edu, BMI, SS, alcohol, and food frequency (green vegetables, soy, milk, beef, and pork)
Mazdak, 2012	2005-2009	Iran	95	95	190	Cases: 73.1 (7.5) Controls: 67.9 (8.3)	Case-Control	Tomato sauce: Median 10	N/A
Mills, 1989	1976-1982	USA, California	180	—	15,000	Cases: ~74	Cohort	Tomato foods: T1<104, T3 ≥520	Age, education, nutrients
Norrish, 2000	1996-1997	New Zealand, Auckland	317	480	797	40-81	Case-Control	Raw tomatoes: Q1 < 91 Q4 >245 Tomato foods: Q1 < 130.9, Q4 >449.4	Age, height, total anti-inflammatory drugs, and SES
Salem, 2011	2005-2008	Iran	194	317	511	Cases: 71.1 (7.8) Controls: 66.5 (10.2)	Case-Control	Tomato foods: T1 ≤ 10 T3 >100	Age, energy, BMI, occupation, SS, alcohol, FHPC

Table 2.1: Characteristics of included studies (continued).

Source Author, Year	Study Period	Country	Number of cases	Number of controls	Total no. participants in cohort	Age (SD)	Study Type	Tomato exposure and dose (grams/week)	Adjustments
Shahar, 2011	2009	Malaysia	35	70	105	Cases: 67.6 (4.7) Controls: 67.8 (4.6)	Case-Control	Tomato sauce: Q1<15.7 Q2 >15.7	Age, race, FHPC, and energy
Sonoda, 2004	1996-2002	Japan	140	140	280	Cases: 59-73	Case-Control	Tomato foods: Q1 ≤199.5, Q4 ≥700	Age, SS and energy
Stram, 2006	1993-1996	USA	3,922	—	82,486	45-75	Cohort	Tomato sauce†, cooked tomatoes†, raw tomatoes†, pizza†	Age, BMI, Edu, FHPC
Subahir, 2009	2003-2008	Malaysia	112	112	224	50-86	Case-Control	Tomato foods†	
Takachi, 2010	1990-2004	Japan	339	—	43,475	45-74	Cohort	Tomato foods: Q1< 42, Q4 >476	Age, location, BMI, SS, alcohol, nutrients, MV, marital status, and screening exams
Villeneuve, 1999	1994-1997	Canada	1,623	1,623	3,246	50-75	Case-Control	Tomato foods: Q1 <104, Q4 ≥728	Age, location, SS, BMI, nutrients, FHPC
Vlajinac, 2010	1990-1994	Serbia and Montenegro	101	202	303	Cases: 70.5 Controls: 71.5	Case-Control	Tomato foods: Median 114.3	Energy

Table 2.1: Characteristics of included studies. † signifies that study does not disclose dose for tomato consumption. Abbreviations: tertile (T), quartile/quintile(Q), years (yrs), body mass index (BMI), family history of prostate cancer (FHPC), Prostate Specific Antigen (PSA), physical activity (PA), education (Edu), smoking status (SS), multivitamin (MV), socioeconomic status (SES), supplemental (supp), treatment (trt), cardiovascular disease (CVD), diagnosis (diag)

Table 2.2. Quality assessment of included studies

Source	Selection				Comparability[5]	Exposure			Total[9]
Author, Year	Definition[1]	Representative	Selection	Definition[4]		Method[6]	Ascertainment[7]	Rate[8]	
Ambrosini, 2008	0	★	0	0	★	★	★	★	5
Bonsetti, 2000	★	★	0	★	★	0	★	0	5
Bonsetti, 2004	★	★	0	★	★★	0	★	★	7
Cohen, 2000	★	0	★	0	★★	0	★	★	6
Darlington, 2007	★	0	★	0	★★	0	★	0	5
Diallo, 2016	★	★	0	★	★★	★	★	★	8
Er, 2014	★	★	0	★	★★	★	★	★	8
Gallus, 2006	★	★	0	★	★	0	★	★	6
Giovannucci, 1995	★	★	0	★	★★	★	★	★	8
Giovannucci, 2007	★	★	0	★	★★	★	★	★	8
Graff, 2016	★	★	0	★	★★	★	★	★	8
Hardin, 2011	★	★	0	★	★★	★	★	0	7
Hodge, 2004	★	★	★	0	★★	★	★	★	8
Jain, 1999	★	★	★	0	★	★	★	0	6
Jian, 2005	★	★	0	★	★★	0	★	★	7
Key,1997	0	★	★	★	★	0	★	★	6
Kirsh, 2006	★	★	0	★	★★	★	★	★	8
Kolonel, 2000	★	★	★	★	★	0	★	0	6
Li, 2008	★	★	★	★	★★	0	★	★	8
Mazdak, 2012	★	★	0	★	0	0	★	0	4
Mills, 1989	★	★	0	0	★	★	★	0	5
Norrish, 2000	★	★	★	★	★	0	★	★	7
Salem, 2011	★	★	0	★	★★	0	★	★	7
Shahar, 2011	★	★	0	★	★★	0	★	0	6
Sonoda, 2004	★	★	0	★	★	0	★	0	5
Stram, 2006	★	★	0	★	★★	★	★	★	8
Subahir, 2009	★	★	0	★	★	0	★	0	5
Takachi, 2010	★	★	0	★	★	★	★	★	7
Villeneuve, 1999	0	★	★	★	★★	0	★	★	7
Vlajinac, 2010	★	★	0	★	★	0	★	0	5

1 indicates that cases are independently validated for case-control studies (0,1 star); 2, cases are from a representative population or drawn from the same community as the nonexposed cohort (0,1); 3, community controls or structured interview for cohort studies (0,1); 4, controls have no history of prostate cancer (endpoint) (0,1); 5, study controls for most important factor (age) and family history of prostate cancer (0, 1, 2); 6, structured interview where blind to case/control status for case-control studies, record linkage or independent blind assessment for cohort studies (0,1); 7, same method of ascertainment for cases and controls or follow-up was long enough for outcomes to occur (4 years) (0,1); 8, same non-response rate or <20% lost to follow up (0,1); 9, total: minimum equals 1; maximum equals 9 stars

Table 2.3: Subgroup analysis of tomato consumption and prostate cancer risk.

	Total Tomato Consumption			Tomato Foods			Cooked Tomatoes and Sauces			Raw Tomatoes		
	No. of studies	RR (95% CI)	P-value	No. of studies	RR (95% CI)	P-value	No. of studies	RR (95% CI)	P-value	No. of studies	RR (95% CI)	P-value
Overall model	26	0.81 (0.71-0.92)†	**0.001**	18	0.84 (0.72-0.98)†	**0.030**	10	0.84 (0.73-0.98)†	**0.029**	10	0.96 (0.84-1.09)†	0.487
Study type												
Case-Control	19	0.68 (0.55-0.84)†	**<0.001**	13	0.69 (0.53-0.91)†	**0.008**	6	0.63 (0.40-1.00)†	0.052	8	0.95 (0.76-1.19)†	0.729
Cohort/NCC	7	0.92 (0.86-0.98)	**0.013**	5	1.00 (0.87-1.15)†	0.963	4	0.92 (0.85-0.99)	**0.025**	2	0.96 (0.81-1.14)†	0.557
Continent												
North America	9	0.95 (0.83-1.10)†	0.501	6	0.97 (0.77-1.22)†	0.800	5	0.92 (0.86-0.99)	**0.033**	5	1.02 (0.88-1.18)†	0.804
Europe	7	0.82 (0.68-0.98)†	**0.029**	5	0.92 (0.76-1.13)†	0.424	2	0.70 (0.40-1.21)†	0.202	4	0.80 (0.68-0.94)	**0.008**
Other	10	0.48 (0.31-0.73)†	**0.001**	7	0.55 (0.35-0.87)†	**0.010**	3	0.20 (0.04-1.10)†	0.064	1	1.04 (0.60-1.80)	0.889
Adjustments												
High quality	13	0.86 (0.76-0.98)†	**0.018**	11	0.86 (0.72-1.03)†	0.093	3	0.93 (0.86-0.996)	**0.039**	5	0.91 (0.77-1.08)†	0.298
Mid-quality	13	0.70 (0.52-0.94)†	**0.017**	7	0.79 (0.54-1.15)	0.211	7	0.66 (0.45-0.96)†	**0.031**	5	1.06 (0.89-1.27)†	0.519
Adjusts for age	22	0.84 (0.74-0.95)†	**0.006**	16	0.88 (0.75-1.03)†	0.101	8	0.91 (0.84-0.97)†	**0.006**	9	0.95 (0.83-1.09)†	0.463
No adjustment for age	4	0.47 (0.22-0.98)†	**0.044**	2	0.49 (0.30-0.80)	**0.005**	2	0.25 (0.01-4.24)†	0.335	1	1.06 (0.62-1.82)	0.833
Adjusts for BMI	14	0.86 (0.72-1.01)†	0.062	10	0.88 (0.69-1.12)†	0.293	5	0.91 (0.85-0.98)	**0.012**	7	0.94 (0.80-1.10)†	0.436
No adjustment for BMI	12	0.74 (0.61-0.90)†	**0.002**	8	0.80 (0.68-0.94)†	**0.006**	5	0.58 (0.32-1.05)†	0.071	3	1.03 (0.78-1.37)	0.830
Adjusts for smoking	10	0.89 (0.76-1.03)†	0.111	9	0.88 (0.72-1.07)†	0.206	2	0.90 (0.82-1.00)	**0.049**	2	0.88 (0.63-1.23)†	0.465
No adjustment for smoking	16	0.73 (0.60-0.89)†	**0.002**	9	0.78 (0.60-1.01)†	0.062	8	0.74 (0.55-0.98)†	**0.037**	8	0.98 (0.84-1.14)	0.808
Adjusts for FHPC	13	0.88 (0.75-1.02)†	0.090	9	0.90 (0.73-1.11)†	0.323	5	0.92 (0.86-0.99)	**0.028**	6	0.97 (0.83-1.14)	0.692
No adjustment for FHPC	13	0.71 (0.56-0.89)†	**0.003**	9	0.85 (0.73-0.98)	**0.021**	5	0.65 (0.41-1.02)†	0.061	4	0.91 (0.71-1.16)†	0.429

Table 2.3: Continued

	Total Tomato Consumption			Tomato Foods			Cooked Tomatoes and Sauces			Raw Tomatoes		
	No. of studies	RR (95% CI)	P-value	No. of studies	RR (95% CI)	P-value	No. of studies	RR (95% CI)	P-value	No. of studies	RR (95% CI)	P-value
Adjusts for energy	15	0.81 (0.67-0.967)†	**0.020**	11	0.82 (0.64-1.04)†	0.101	6	0.88 (0.80-0.97)	**0.008**	4	1.05 (0.91-1.21)	0.485
No adjustment for energy	11	0.80 (0.67-0.96)†	**0.016**	7	0.86 (0.69-1.07)†	0.169	4	0.74 (0.45-1.16)†	0.189	6	0.91 (0.76-1.09)†	0.289
Adjusts for education	9	0.83 (0.65-1.08)†	0.167	8	0.85 (0.63-1.14)†	0.270	2	0.95 (0.85-1.06)	0.345	4	0.98 (0.77-1.25)†	0.884
No adjustment for education	17	0.79 (0.69-0.91)†	**0.001**	10	0.83 (0.70-0.98)†	**0.025**	8	0.77 (0.61-0.97)†	**0.026**	6	0.93 (0.83-1.05)	0.263
Adjusts for PA	4	0.97 (0.72-1.31)†	0.326	2	1.14 (0.80-1.62)	0.479	2	0.91 (0.82-1.00)	0.049	3	0.84 (0.67-1.05)†	0.126
No adjustment for PA	22	0.75 (0.64-0.88)†	**<0.001**	16	0.79 (0.66-0.95)†	**0.010**	8	0.74 (0.55-0.98)†	**0.037**	7	1.06 (0.97-1.16)	0.207
Adjusts for alcohol	5	0.88 (0.62-1.24)†	0.459	4	0.80 (0.44-1.46)†	0.468	1	0.89 (0.80-1.00)	**0.043**	1	0.74 (0.58-0.94)	**0.012**
No adjustment for alcohol	21	0.78 (0.68-0.90)†	**0.001**	14	0.83 (0.71-0.99)†	**0.032**	9	0.80 (0.64-0.99)	**0.056**	9	1.01 (0.94-1.09)	0.283

† signifies that results are estimated by DerSimonian-Laird random effects model. Bolded entries signify a significant result p<0.05. Abbreviations: relative risk (RR), number (No.), body mass index (BMI), family history of prostate cancer (FHPC), Nested Case-Control (NCC).

Figure 2.1 Literature search and study selection flow chart.

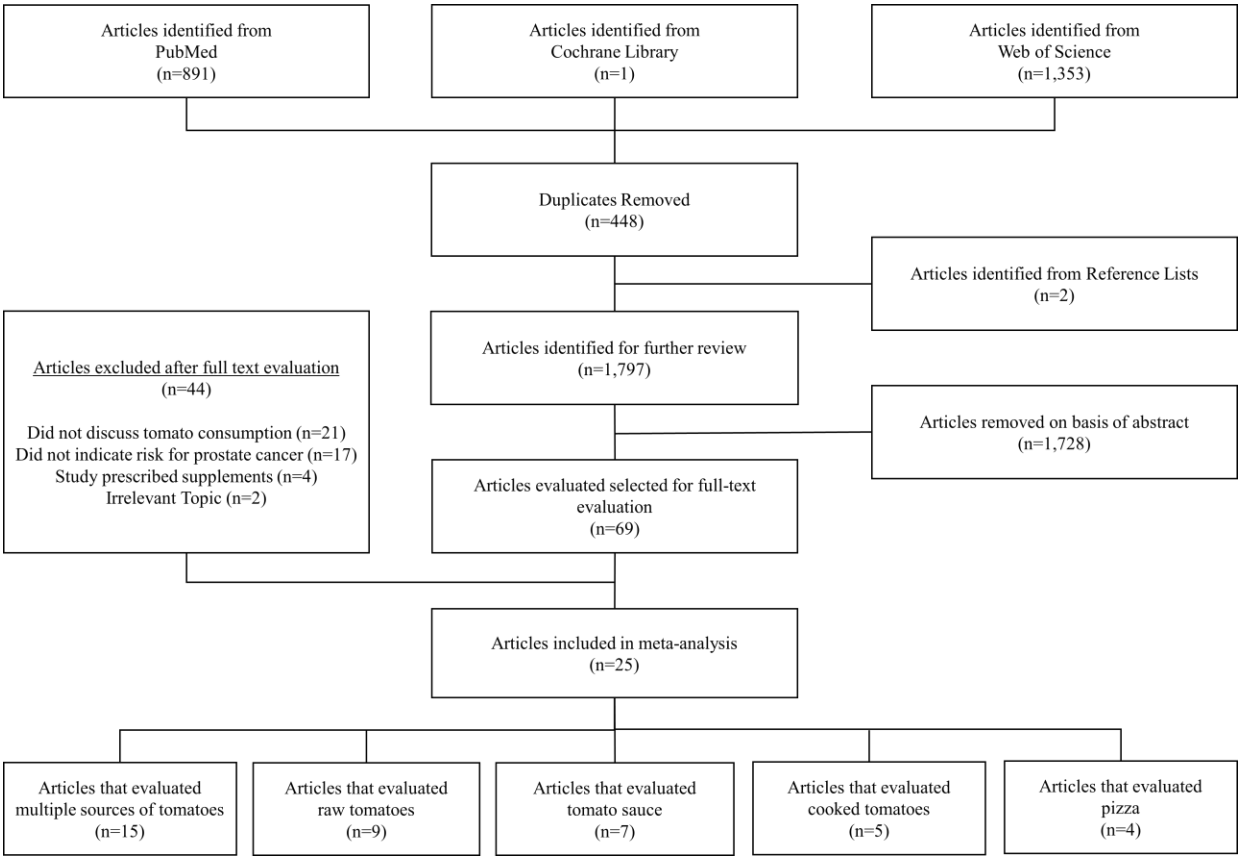
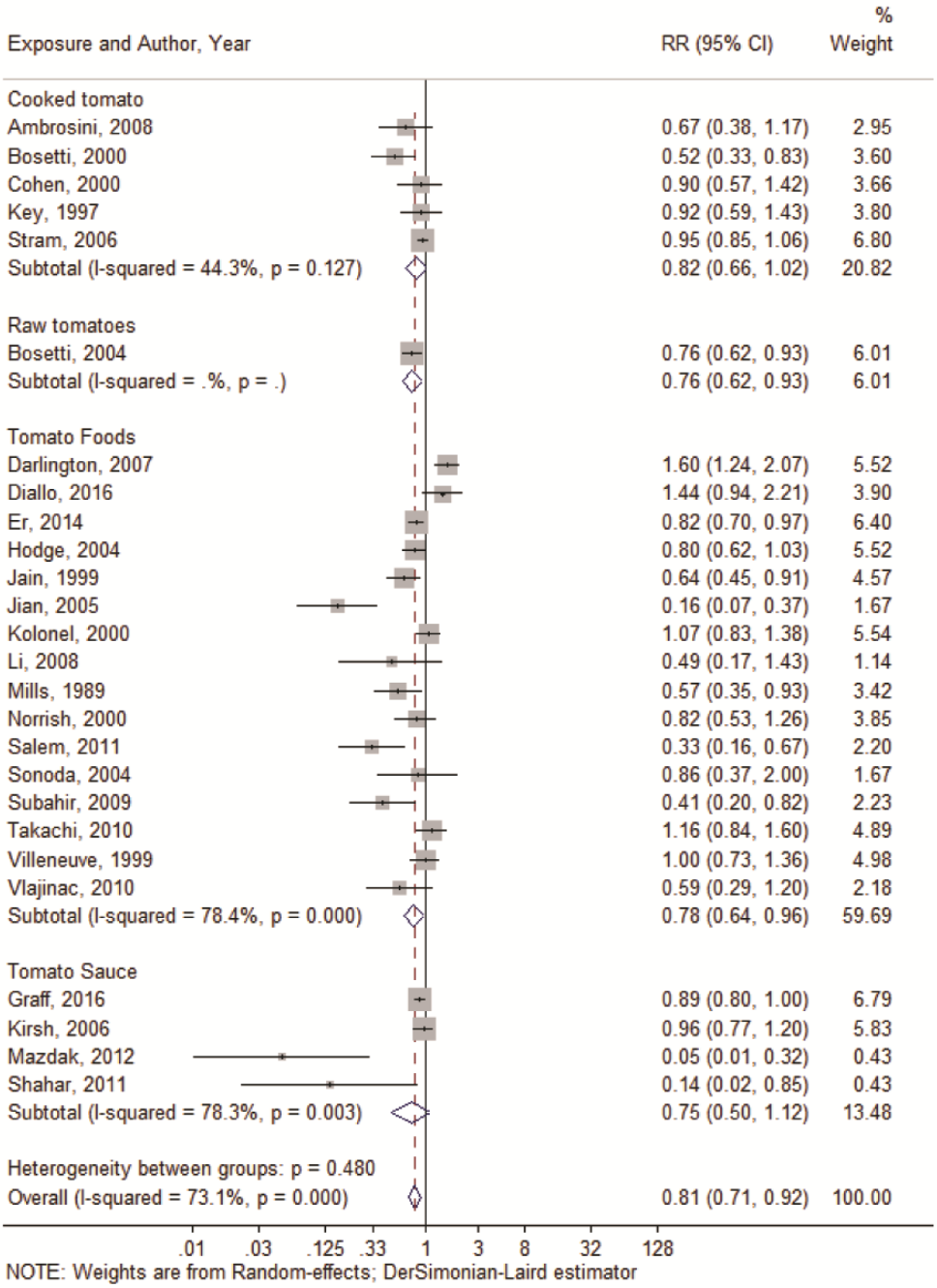
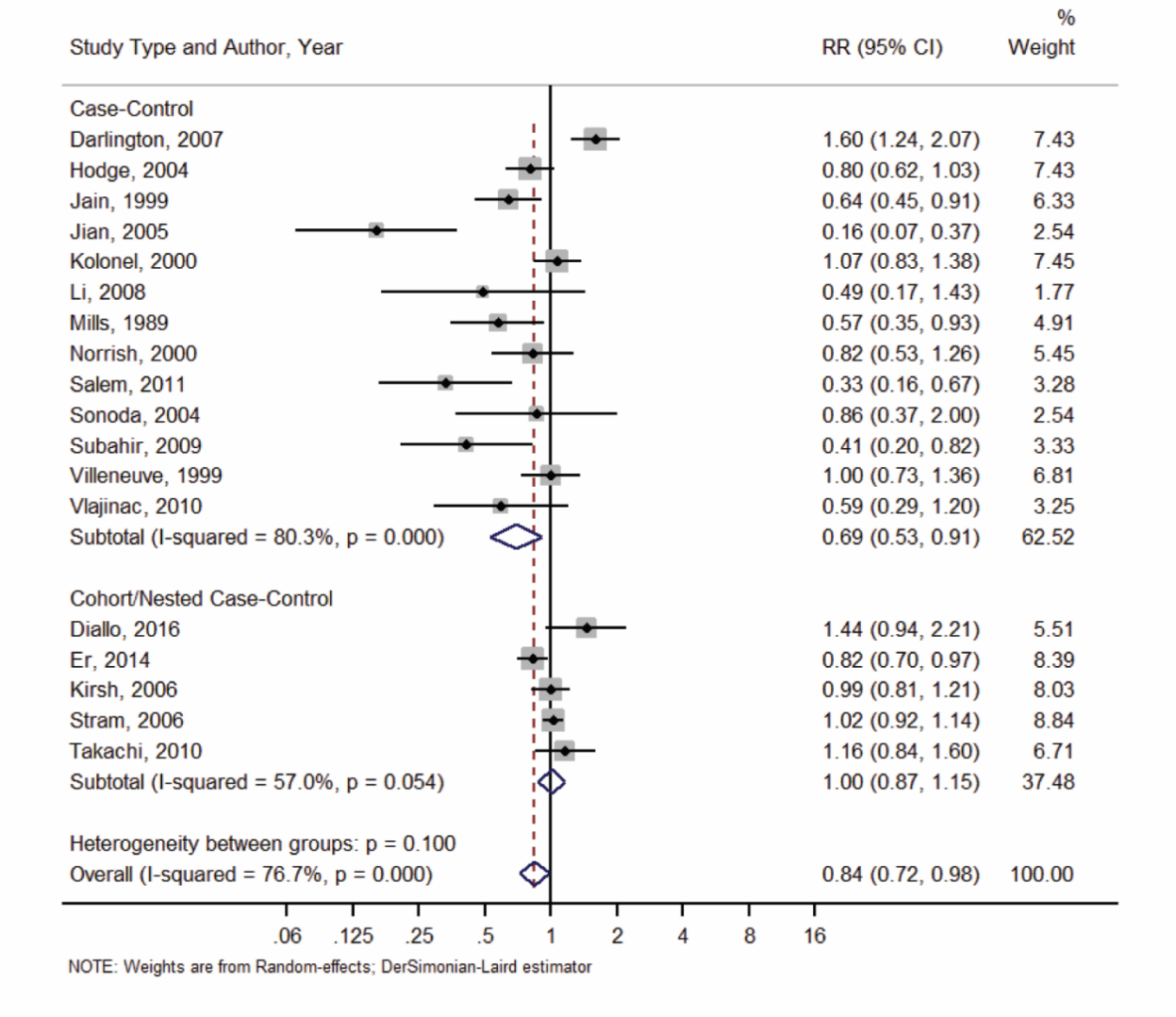


Figure 2.2: Forest plot for association of highest vs lowest total quantile of tomato consumption on PCa risk.



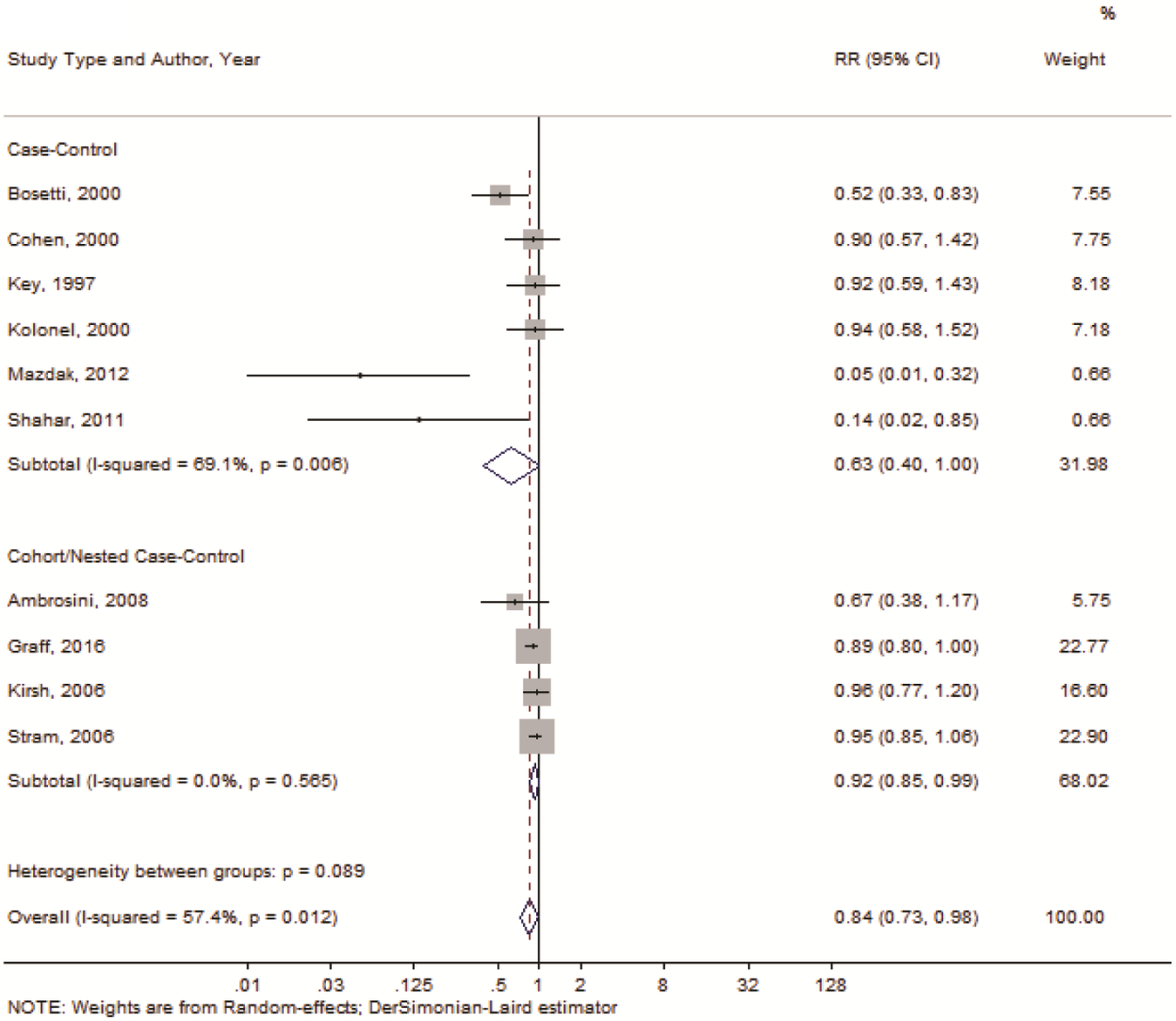
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 2.3 Forest plot for association of highest vs lowest quantile of tomato foods on PCa risk.



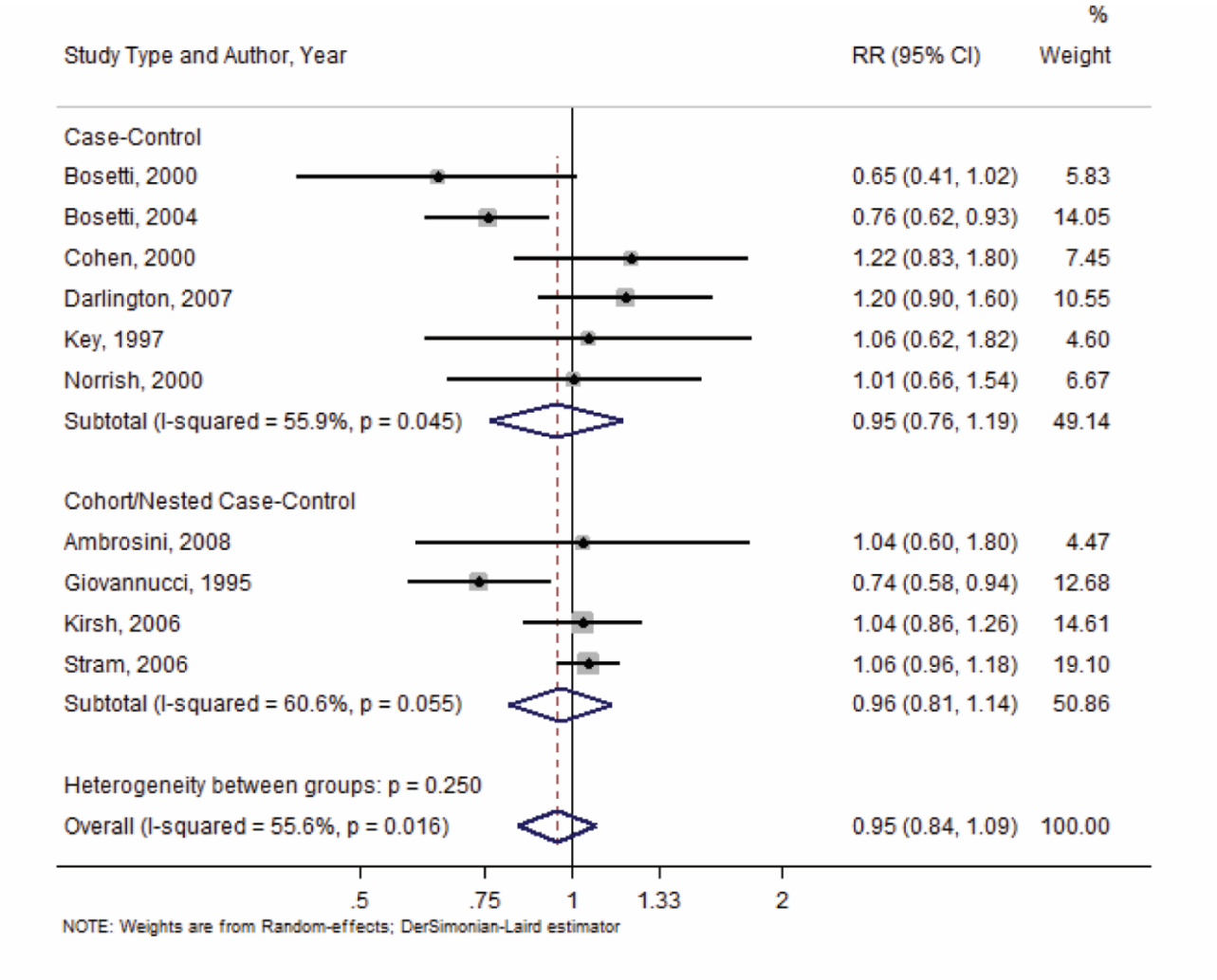
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 2.4 Forest plot for association of highest vs lowest quantile of cooked tomatoes and sauces on PCa risk.



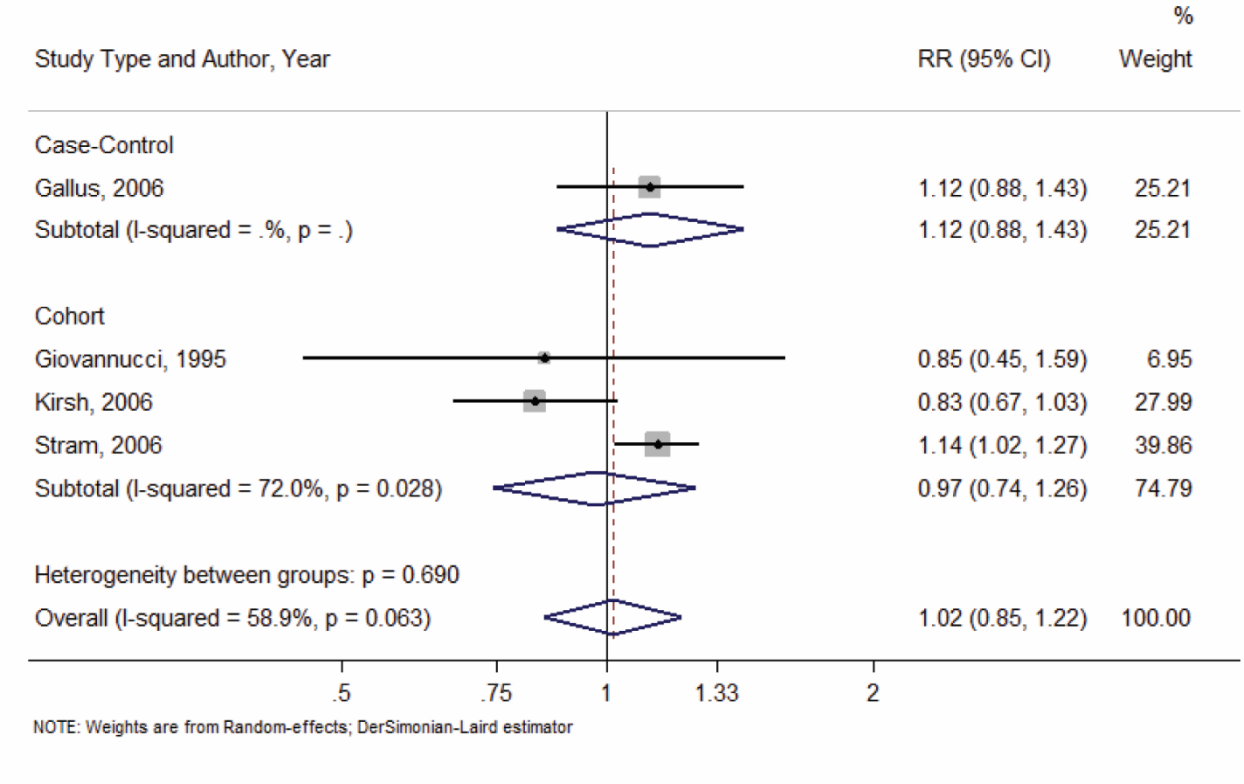
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 2.5 Forest plot for the association of highest vs. lowest quantile of raw tomatoes on PCa risk.



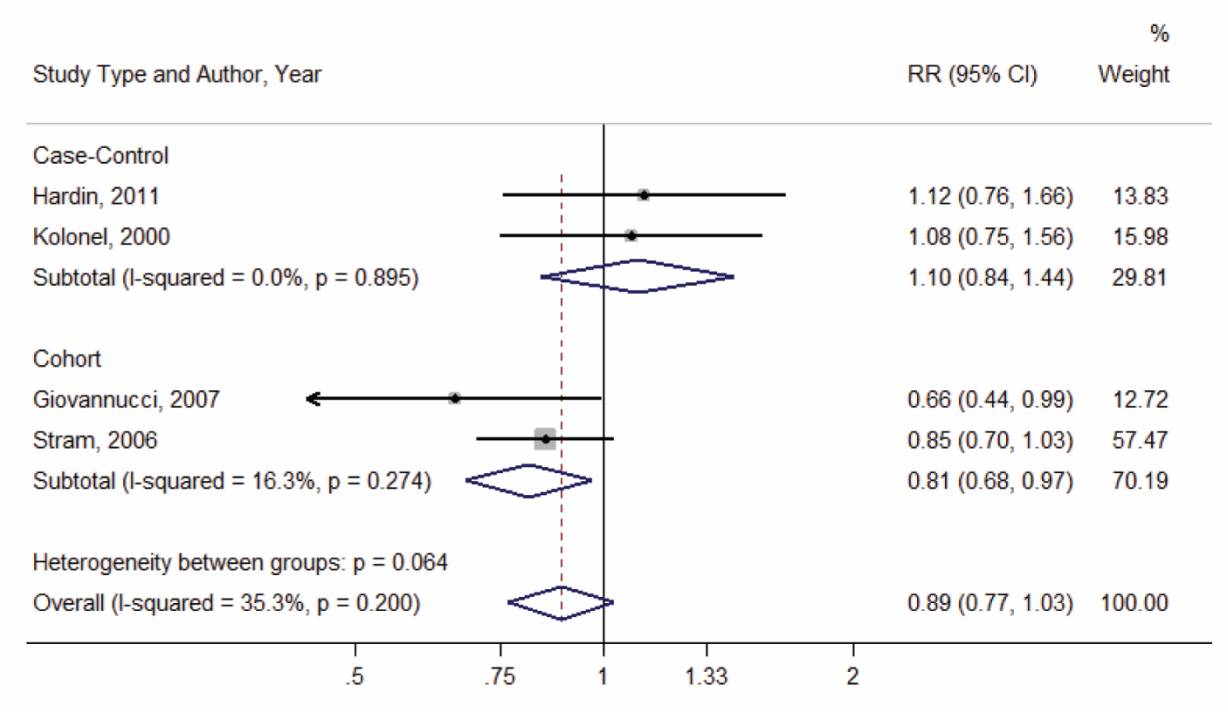
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 2.6 Forest plot for the association of highest vs lowest quantile of pizza on PCa risk.



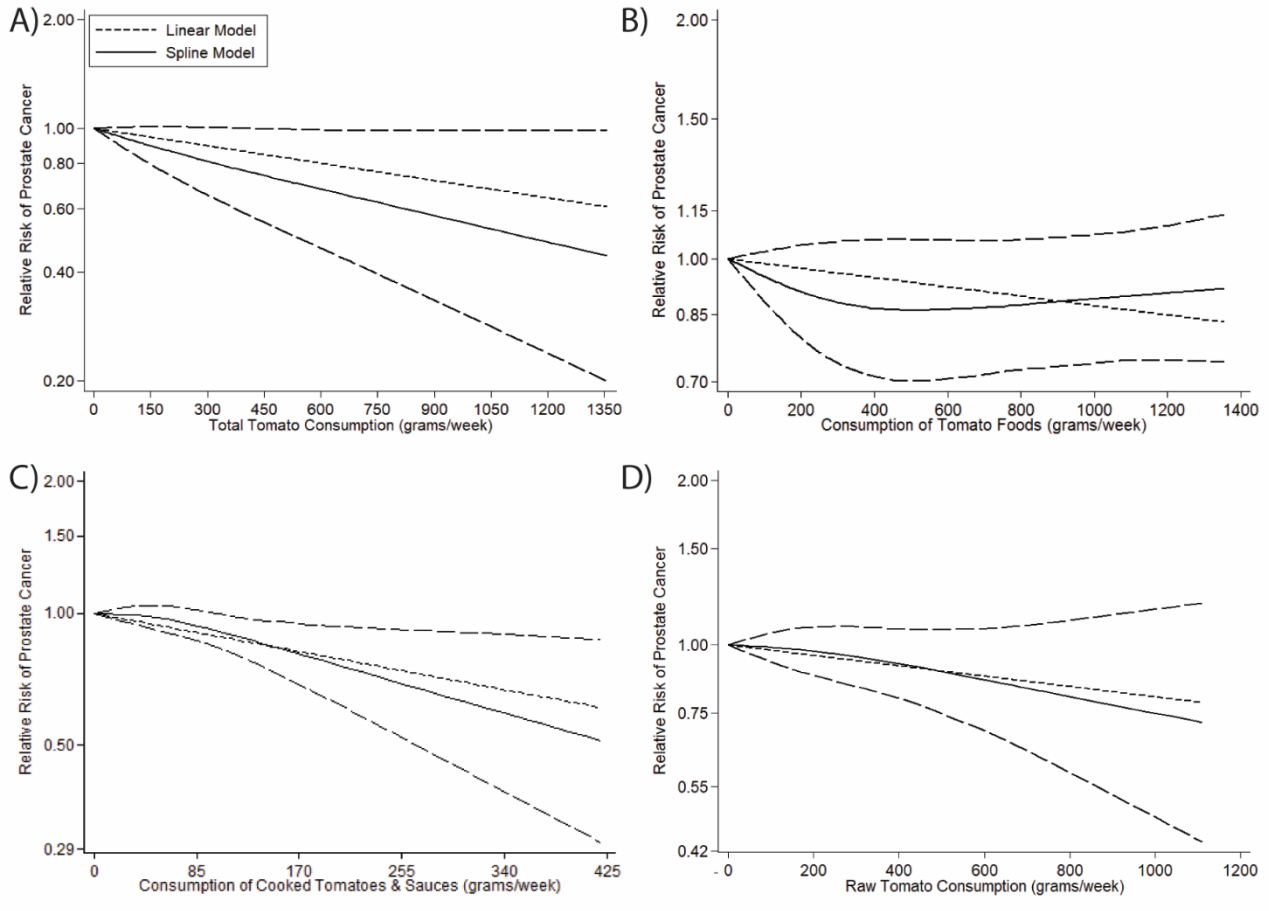
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 2.7 Forest plot for the association of highest vs lowest quantile of tomato consumption on advanced PCa risk.



This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 2.8 Dose-response analysis of tomato consumption and PCa risk.



The solid black line is the nonlinear model curve for published relative risk and the two dotted lines are the corresponding 95% confidence intervals. The short-dashed line is the linear model curve for the published relative risks (RR); (A) total tomato consumption and risk of PCa after exclusion of one study; (B) tomato foods and risk of PCa after exclusion of one study (C) cooked tomatoes and sauces and risk of PCa (D) raw tomatoes and risk of PCa.

Chapter 3: **Increased dietary and circulating lycopene are associated with reduced prostate cancer risk: a systematic review and meta-analysis**

[b] *Abstract*

Background: Prostate cancer (PCa) is the fifth leading cause of cancer-related deaths worldwide. Many epidemiological studies have investigated the association between prostate cancer and lycopene; however, results have been inconsistent. We hypothesized that dietary and circulating concentrations of lycopene would be inversely associated with PCa risk.

Methods: We conducted a systematic review and dose-response meta-analysis for the association between dietary and circulating lycopene and PCa risk. Eligible studies were published before December 1, 2016 and were identified from PubMed, Web of Science, and the Cochrane Library. We estimated pooled relative risk ratios (RR) and 95% confidence intervals using random and fixed effects models. Linear and nonlinear dose-response relationships were also evaluated for PCa risk.

Results: Forty-two studies were included in the analysis, which included 43,851 cases of PCa reported from 692,012 participants. Both dietary intake (RR=0.88, 95% CI: 0.78-0.98, p=0.017) and circulating concentrations (RR=0.88, 95% CI: 0.79-0.98, p=0.019) of lycopene were associated with reduced PCa risk. Sensitivity analyses within the dose-response analysis further revealed a linear dose-response for dietary lycopene and PCa risk such that PCa decreased by 1% for every additional 2 mg of lycopene consumed

[b] The content of this chapter has been published (Reference 132. Rowles III JL, Ranard KM, Smith JW, An R, Erdman Jr JW. Increased dietary and circulating lycopene are associated with reduced prostate cancer risk: a systematic review and meta-analysis. *Prostate Cancer And Prostatic Diseases.* 2017;20:361.).

(p=0.026). Additionally, PCa risk decreased by 3.5% to 3.6% for each additional 10 µg/dL of circulating lycopene in the linear and nonlinear models respectively (p_{linear}=0.004, $p_{nonlinear}$=0.006). While there were no associations between lycopene and advanced PCa, there was a trend for protection against PCa aggressiveness (RR=0.74, 95% CI: 0.55-1.00, p=0.052).

Conclusions: Our data demonstrate that higher dietary and circulating lycopene concentrations are inversely associated with PCa risk. This was accompanied by dose-response relationships for dietary and circulating lycopene. However, lycopene was not associated with a reduced risk of advanced PCa. Further studies are required to determine mechanisms underlying these associations.

Introduction

Prostate cancer is the second most common cancer in men and is the fifth leading cause of cancer-related deaths worldwide[1]. The development of PCa is complex and influenced by a combination of genetic, hormonal and environmental factors, including diet[97]. Diet is a highly modifiable risk factors for PCa, and epidemiological evidence indicates that consumption of tomato products is associated with a reduced risk of PCa[94,224,225]. Lycopene, a lipophilic carotenoid that gives tomatoes their red color, is believed to be the primary bioactive component in tomatoes, and tomatoes are the source of more than 85% of dietary lycopene in the United States[44,45]. Despite potential benefits seen in the literature, epidemiological studies about lycopene and its role in prostate carcinogenesis and progression have yielded mixed results.

Previously, four meta-analyses have been conducted to investigate the associations between tomatoes or lycopene and the risk of PCa[128-131]. Of these analyses, three have shown an inverse association with elevated circulating lycopene[129-131], and one showed an inverse association with dietary lycopene[130]. Since these studies were published, new evidence has surfaced that solicits a closer investigation of the association between lycopene and PCa risk. The current study aimed to update previous analyses by systematically and quantitatively evaluating the association between lycopene and PCa risk. Furthermore, we utilized dose-response meta-analyses to assess potential dose-responses, as well as cumulative meta-analyses to display trends over time.

Materials and methods

(Parallel statistical approaches were used in Meta-analysis 1 and 2. Only novel aspects of the methodology for this are described below)

Study selection criteria

This meta-analysis was conducted in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines[138,139]. Studies that met the following criteria were included in the meta-analysis: (a) evaluated the association between lycopene and PCa risk by using randomized control trials, cohort, cross-sectional, retrospective, prospective, and case-control studies; (b) methodology was documented in replicable detail; (c) evaluated the relationship between lycopene and prostate cancer risk; (d) included relative risk ratios (RR) with 95% confidence intervals

for exposure categories; (e) were written in English; and (f) peer-reviewed publications or theses. Among studies that met the above criteria, those that provided information about doses of lycopene, length of intervention, and relative risk for primary or advanced PCa were included in the dose-response analysis.

Literature search

We conducted a comprehensive literature search of PubMed, Web of Science and the Cochrane Library using the following key words: prostate cancer, prostate neoplasm, lycopene, tomato, carotenoids, humans, case-control studies, follow-up studies, cohort studies, prospective studies and their variants (up to December 1st, 2016). Titles and abstracts of articles that were identified by the keyword search were screened against the study selection criteria. Potentially relevant articles were retrieved for evaluation of the full text. We also conducted a reference list search (i.e., backward search) and cited reference search (i.e., forward search) from full text articles meeting the study selection criteria. Articles identified through this process were further screened and evaluated using the same criteria. We repeated searches on all newly identified articles until no further relevant articles were found. Two authors (JR3 and KR) individually determined inclusion/exclusion of all articles retrieved in full text and discrepancies were resolved through discussion.

Statistical analysis

STATA/IC version 14.1 (StataCorp LP, College Station, TX) was utilized to analyze the data. Because studies reported different exposure categories as tertiles, quartiles or quintiles, we used the study-specific RR for the highest quantile compared to the lowest quantile of dietary lycopene intake and/or circulating (serum or plasma) lycopene

concentrations. If the total number of cases or person-years were presented without distribution, we estimated the distribution by dividing by the number definitions of the quantiles. If the unit for circulating concentrations was reported as μmol/L, it was multiplied by 536.89 (molecular weight of lycopene) and adjusted to μg/dL.

For the meta-analysis of the dose-response relationship between lycopene and PCa risk, the method proposed by Greenland and Longnecker of generalized least squares for trend estimation was utilized[150,151]. Based on the dose of lycopene intake or circulating blood concentration provided for each quantile, study-specific slopes (with 95% CIs) were generated. If a range was provided for lycopene exposure rather than a median, the lower boundary of the quantile was utilized. We examined linear and nonlinear associations between PCa risk and lycopene intake or circulating concentrations of lycopene by plotting linear and nonlinear dose-response curves using restricted cubic spines, with 3 knots at 10%, 50% and 90% of the distribution. A p-value for curve linearity and nonlinearity was calculated by testing that the coefficient of the second spline was equal to zero. $p<0.05$ was considered statistically significant for all analyses.

Results

Literature search

The initial abstract screening and reference list search yielded a total of 2,208 articles after removing duplicates. After screening these titles and abstracts, 95 articles remained for full-text evaluation. Among these articles, 41 were included and 54 were excluded. Among the 54 excluded articles, 24 did not evaluate the risk for PCa[76,226-248],

13 had an irrelevant topic[96,153,179,181,193,233,249-254], 12 did not indicate lycopene status[175,202,206,255-263], 3 studies had a shorter follow-up period compared to the included study[191,217,220], and 2 used the same data found in other included studies[94,200]. From the initial 41 articles that were included in the meta-analysis, 42 studies evaluated associations between lycopene and prostate cancer (one article reported results from two cohorts[264]). Among these studies, 25 reported the dietary associations with lycopene[8,95,152,154,159,162,163,198,199,201,205,208,214,216,218,222,265-273] and 18 reported associations with circulating lycopene[60,171,264,268,274-286] on PCa (Figure 3.1).

Study Characteristics

Table 3.1 summarizes the 25 studies that investigated dietary lycopene, and Table 3.2 summarizes the 18 studies that investigated circulating lycopene. Among these studies, 19 were case-control[154,159,162,163,198,199,205,214,216,218,222,265,267-269,271,275,285,286], 13 were nested case-control[171,264,266,274,276-279,281-284], 8 were cohort[8,60,95,201,208,270,273,280], and 2 were case-cohort studies[152,272]. These studies included a combined total of 43,851 PCa cases reported from 692,012 participants. Thirty-two studies were from North America[8,60,95,152,154,159,163,171,199,201,216,222,264,266-271,273,275-279,282-286], 6 were from Europe[205,214,218,272,280,281], 2 were from Australia[198,274], 2 were from Asia (China and Singapore)[162,222], and 1 was from South America (Uruguay)[265]. Many of the studies provided risk estimates that were adjusted for age (n=34)[8,60,95,152,154,159,162,163,198,199,201,205,214,216,222,264-266,268-276,278-280,282,284-286], body mass index (n=24)[8,95,152,154,159,162,163,171,199,201,208,214,216,264-267,276,277,279,281,282,286], smoking (n=19)[8,60,95,154,163,171,199,201,264,267,268,270,273,275,278-281,286], education (n=18)[8,152,154,162,199,208,214,216,264,265,267-269,273,277,280,281,286], family history of prostate cancer

(n=18)[8,95,171,198,201,208,214,216,222,265,267-272,277,280], physical activity (n=10)[8,95,152,201,214,273,276,279-281], or alcohol intake (n=9)[8,267,268,270,276,279-281]. All studies that reported intake values utilized a food frequency questionnaire (1 study adapted a food frequency questionnaire that was used in the form of an interview[218]), and all studies reporting circulating lycopene values used high performance liquid chromatography (HPLC) for quantification of carotenoids. Three studies defined advanced PCa with Gleason scores[266,277,282], 2 used stage[208,272], and 6 used a combination of the two[152,201,276,281,284,285].

The Newcastle-Ottawa Scale was utilized to assess quality of all included studies. As described in Table 3.3, the highest quality score was 9 and the lowest was 6. The average quality score was 8.26 (SD: 0.81) for case-control studies, 8.70 (SD: 0.67) for cohort studies, and 8.46 (SD: 0.78) for nested case-control studies. Overall, the mean quality score was 8.41 (SD: 0.77), suggesting that the majority of included studies were of high quality.

Dietary lycopene and PCa risk

A total of 25 studies reported the relative risk of PCa with lycopene intake[8,95,152,154,159,162,163,198,199,201,205,208,214,216,218,222,265-273]. Of these studies, 16 were case-control studies [154,159,162,163,198,199,205,214,216,218,222,265,267-269,271], 8 were cohort/case cohort studies[8,95,152,201,208,270,272,273], and 1 was a nested case-control study[266]. All of these studies had complete relative risk information and were included in the meta-analysis. Figure 3.2 demonstrates the pooled RR of PCa when comparing the highest lycopene intake to the lowest. The pooled RR for this comparison was 0.88 (95% CI: 0.78-0.98, p=0.017), indicating a reduced risk for PCa with high lycopene intake. Specifically, the

pooled RR was 0.93 (95% CI: 0.87-0.99, p=0.030) for cohort studies and 0.82 (95% CI: 0.68-0.999, p=0.049) for case-control studies. Furthermore, we investigated publication bias by funnel plot (Figure C.1A), Begg's correlation test and Egger's linear regression test. Begg's correlation test (p=0.032) was significant, whereas Egger's liner regression test (p=0.130) for bias was nonsignificant.

We further conducted a series sensitivity and subgroup analyses. A statistically significant result was first achieved in 2005 and remained unchanged after 8 additional studies were published (Figure C.2). The heterogeneity between these studies was predominately due to one study from China[162]. After this study was excluded, the overall heterogeneity I^2 decreased from 54.1% to 31.3%. Begg's correlation test (p=0.112) and Egger's linear regression test (p=0.329) also became nonsignificant. Removal of this study from the dataset did not substantially affect the overall pooled RR (Figure C.3, RR= 0.91, 95% CI: 0.83-0.99, p=0.034). We also investigated the influence of study type, location and covariate adjustments on the pooled RR. Table 3.4 describes subgroup analysis for dietary lycopene on PCa risk. Studies conducted in North America and continents other than Europe yielded an overall significant RR of 0.93 (95% CI: 0.87-0.99, p=0.036) and 0.59 (RR=95% CI: 0.35-0.99, p=0.046) respectively, while studies conducted in Europe (RR=0.93, 95% CI: 0.79-1.10, p=0.389) did not display an association between lycopene consumption on PCa incidence. High-quality studies also displayed a borderline significant protective association (RR=0.89, 95% CI: 0.79-1.00, p=0.051). Studies that adjusted their models for age (p=0.007) demonstrated protective associations with increased lycopene intake. Additionally, the pooled RR was not affected

by studies that prescribed supplements to their study population compared to studies that did not (Figure C.4, RR=0.88, 95% CI: 0.79-0.99, P=0.030).

With respect to the associations of lycopene and advanced PCa, Figure 3.3 shows the pooled association for the highest intake of lycopene compared to the lowest. This association was not significant, (RR=0.93, 95% CI: 0.82-1.07, p=0.307) and had low within-study heterogeneity (I^2=12.8%). Figure C.1C displays the funnel plot for publication bias in studies investigating advanced PCa. Begg's correlation test (p=0.806) and Egger's liner regression test (p=0.646) for bias were both nonsignificant. There was no significant effect of time (Figure C.5) and there were no studies that prescribed supplements to their study.

Circulating lycopene and PCa risk

There were 18 studies that investigated relationships between circulating blood concentrations of lycopene and PCa[60,171,264,268,274-286]. Of these studies, 2 were cohort studies[60,280], 4 were case-control studies [268,275,285,286] and 12 were nested case-control studies[264,274,276-279,281-284]. All of these studies had complete relative risk information and were included in the meta-analysis.

Figure 3.4 shows the pooled RR of the highest circulating lycopene quantile compared to the lowest quantile. The pooled RR of highest compared to lowest category of circulating lycopene was 0.88 (95% CI: 0.79-0.98, p=0.019). The RR for high vs. low circulating lycopene for cohort studies was 0.78 (95% CI: 0.37-1.65, p =0.516), case-control studies yielded a RR of 0.60 (95% CI: 0.32-1.13, p=0.115), and nested case-control studies yielded a RR of 0.91 (95% CI: 0.81-1.02, P=0.093). There was low heterogeneity between studies (I^2 = 26.2%). Publication bias was explored through a funnel plot (Figure C.1B),

Begg's correlation test, and Egger's linear regression test. Begg's correlation test (p=0.064) was nonsignificant and Egger's linear regression test (p=0.013) was significant, indicating potential statistical publication bias.

Next, we conducted several sensitivity and subgroup analyses. Cumulative meta-analyses suggested that a statistically significant result was first achieved in 2001 and remained unchanged after 13 additional studies were published (Figure C.6). There were no studies that strongly influenced the heterogeneity of the dataset; however, one study had a substantially higher range of circulating lycopene[284]. Removal of this did not strongly affect Begg's correlation test (p=0.115) and Egger's linear regression test (p=0.017), but its exclusion improved the pooled RR (Figure C.7, RR= 0.85, 95% CI: 0.76-0.95, p=0.006). Table 3.4 describes the subgroup analysis for associations between circulating lycopene and PCa risk. Studies conducted in North America displayed an inverse relationship between circulating lycopene and PCa (RR=0.87, 95% CI: 0.78-0.98, p=0.025), while studies in Europe (RR=0.94, 95% CI: 0.70-1.26, p=0.670) and other continents did not (RR=0.77, 95% CI: 0.40-1.48, p=0.431). High-quality studies found an association between high lycopene and PCa incidence (p=0.013), while lower-quality studies did not (p=0.946). Adjustments for BMI (p=0.013), smoking (p=0.022), family history of prostate cancer (p=0.005), education (p=0.025), physical activity (p=0.016), and alcohol (p=0.041) revealed associations between circulating lycopene and PCa incidence. The pooled RR was not affected by excluding studies that prescribed dietary supplements to their study population compared to studies that did not (Figure C.8, RR=0.83, 95% CI: 0.69-0.996, P=0.045).

Figure 3.5 displays the pooled association for circulating lycopene in relation to advanced PCa. There was no significant association of lycopene on advanced PCa, (RR=0.77 95% CI 0.50-1.17, p=0.216) and there was considerable heterogeneity across studies (I^2 = 64.1%). The funnel plot for publication bias is displayed in Figure C.1D. Begg's correlation test (p=0.806) and Egger's liner regression test (p=0.485) for bias were both not significant. There was no significant effect of time (Figure C.9). Additionally, the pooled RR was not affected by studies that prescribed dietary supplements to their study population compared to studies that did not (Figure C.10, RR=0.81 95% CI 0.49-1.34, p=0.416).

Aggressiveness and Mortality

Figure 3.6 displays the pooled association of lycopene on PCa aggressiveness and Figure 3.7 displays the pooled association of lycopene on PCa mortality. There were 2 studies that investigated associations between lycopene on aggressiveness[60,154] and 2 studies that investigated the associations of lycopene and mortality[95,273]. For PCa aggressiveness, patients with nonaggressive PCa (Gleason Score <7) were used as the reference group, to which patients with aggressive PCa (stage III or IV or Gleason score ≥ 7) were compared. The pooled RR comparing the highest lycopene to lowest lycopene with respect to PCa aggressiveness approached statistical significance (RR=0.74, 95% CI: 0.55-1.00, p=0.052) with no heterogeneity between studies (I^2=0%). The pooled RR for mortality was not significant (RR=0.85 95% CI 0.62-1.17, p=0.324) and displayed considerable heterogeneity (I^2=69%).

Dose-response

Of the studies eligible for dose-response analysis, 16[8,95,152,159,162,163,198,199,201,205,216,218,265,267,268,272] investigated dietary lycopene with respect to PCa incidence. As seen in Figure 3.8A, there were no significant associations with PCa risk (p_{linear}=0.221, $p_{nonlinear}$=0.153). During sensitivity analyses, one study significantly increased the heterogeneity of the data[162]. Removal of this study resulted in a significant linear dose-response relationship (Figure 3.8B, p_{linear}=0.026, $p_{nonlinear}$=0.096) such that PCa risk decreased by 1% for every additional 2 mg of lycopene consumed (95% CI 0.98-0.999).

Thirteen studies[264,268,274,276-282,284,286] that investigated the association of circulating lycopene on risk of PCa were eligible for the dose-response analysis. Figure 3.8C shows the association between lycopene concentrations and PCa risk (p_{linear}=0.105, $p_{nonlinear}$=0.049). For each additional 10 µg/dL of circulating lycopene, there was a decrease in PCa risk by 3% (95% CI 0.94-1.00) in the nonlinear model. During sensitivity analyses, one study had a significantly higher range of circulating lycopene than the other included studies[284]. Removal of this study significantly improved the model for the dose-response relationship resulting in a significant linear and nonlinear dose-response relationship (Figure 3.8D, p_{linear}=0.004, $p_{nonlinear}$=0.006). For each additional 10 µg/dL of circulating lycopene, there was a 3.5% and 3.6% (linear and nonlinear 95% CI 0.94-0.99) decrease in PCa risk for the linear and nonlinear models respectively.

Fewer studies investigated the association between lycopene and advanced PCa. Four studies[152,201,266,272] that investigated dietary lycopene and 5 studies[276,277,281,282,284] that investigated circulating lycopene were eligible for the dose-response analysis. There

was no significant association between dietary lycopene and advanced PCa (Figure 3.8E, p_{linear}=0.585, $p_{nonlinear}$=0.285). Additionally, no dose-responses were observed between circulating lycopene and risk for advanced PCa (Figure 3.8F, p_{linear}=0.946, $p_{nonlinear}$=0.351).

Discussion

The present systematic review and meta-analysis analyzed 42 studies, which included 43,851 cases of PCa reported from 692,012 participants. We demonstrated an inverse association between PCa risk and higher exposures of both dietary and circulating lycopene. However, elevated exposure of dietary or circulating lycopene did not lower the risk for advanced PCa, mortality or aggressiveness. Cumulative meta-analyses revealed associations between lycopene and PCa risk that had persisted over time. Sensitivity analyses within the dose-response analysis further revealed a significant and novel linear dose-response for dietary lycopene and PCa risk such that PCa decreased by 1% for every additional 2 mg of lycopene consumed. Additionally, we found that PCa risk decreased by 3.5% to 3.6% for each additional 10 µg/dL of lycopene in the circulation in the linear and nonlinear models respectively.

Due to large variation of lycopene content in foods, food frequency questionnaires (FFQ), diet records or diet histories may not be accurately estimate long-term dietary lycopene consumption[287]. There is also a large degree of diversity within questionnaires, and as a result, the lycopene consumption may not be precisely estimated across studies. Potentially due to these issues, the majority of previous meta-analyses have not found a relationship with dietary lycopene[128,129,131]. Despite these factors, we found a significant

inverse association between of dietary lycopene and PCa risk (p=0.017). Additionally, this is the first meta-analysis to find a dose-response with dietary lycopene and PCa risk. This may have resulted from a lack of power. The current analysis included an additional 12 studies that investigated the association between dietary lycopene on PCa risk, nearly doubling the number of studies analyzed from previous analyses.

Circulating blood concentrations of lycopene may be a more reliable and accurate estimation of lycopene intake compared to dietary questionnaires[288]. The majority of meta-analyses have demonstrated protective associations of circulating lycopene and PCa risk[129-131]. Similarly, the current study found an inverse association (p=0.018). Previous meta-analyses have also found a dose-response for circulating lycopene; PCa risk decreased by 2-3% for each additional 10 µg/dL of circulating lycopene[129,131]. In the current study, PCa risk decreased by 3% for each additional 10 µg/dL of lycopene in the circulation. After removing one study[284] with a significantly higher range of circulating lycopene, this association became more significant. PCa risk decreased by 3.5% to 3.6% for each additional 10 µg/dL of circulating lycopene. Combined with previous work, these data support the hypothesis dietary and circulating lycopene reduce risk of PCa.

Subgroup analysis revealed several nuances in the data. Several confounding factors, such as study type, location and adjustments in the regression model displayed significant associations. Cohort studies demonstrated the strongest correlation for dietary lycopene, but there was no difference between study types regarding for circulating lycopene. Additionally, high-quality studies revealed a reduced risk for PCa, whereas medium quality studies did not for studies investigating dietary and circulating lycopene. This association may have been affected by factors related to the study design or

analysis. All of the medium quality studies investigating dietary lycopene did not utilize a validated FFQ, and medium quality studies that investigated circulating lycopene did not include proper covariates in their analyses. Studies that accounted for BMI, smoking, family history of prostate cancer, education, physical activity, and alcohol consumption were associated with a reduced risk of PCa. Studies that did not account for these covariates were not associated with PCa risk.

Although increased dietary and blood lycopene were predictive of reduced risk for total PCa, there was no association observed for advanced prostate cancer. A number of factors may have contributed to this lack of association. Most studies did not include the grade or stage of PCa and contained only a small number of cases with advanced stages of PCa. As a result, the true associations between lycopene and advanced PCa risk may not be identifiable. Key et. al. pooled individual data from several large cohort studies and found an inverse association between circulating and PCa risk[94]. Additionally, when investigating aggressiveness, the pooled effect was 0.74 (95% CI 0.55-1.00, p=0.052), indicating that lycopene may be related to the aggressiveness of PCa. Investigating the relationship between lycopene and PCa at different stages may provide further insight into the role of lycopene in PCa progression.

Our analysis exhibits several strengths that improve upon existing studies in the literature. First, compared to previous analyses, we included 16 additional studies that investigated PCa risk. Cumulative, sensitivity and subgroup analyses were conducted to examine the sources of heterogeneity and evaluate associations between lycopene and PCa. We had several concerns regarding studies that prescribed supplements to their study population. The most commonly prescribed supplements in these studies were

retinyl palmitate and beta-carotene. Absorption of lycopene and beta-carotene occurs in the duodenum of the small intestine and is dependent on Class B Scavenger Receptor (SR-B1) membrane protein[50,51]. Beta-carotene is absorbed more efficiently than lycopene[52], and supplementation may result in reduced lycopene bioavailability. Additionally, a consistent finding from studies that utilized beta-carotene and retinyl palmitate supplements was an increased risk of cancers in conjunction with other behavioral patterns such as smoking[289-291]. In light of this, we also analyzed the studies that did not prescribe supplements to their study. We found no differences between this analysis and the analysis of the studies that included these prescribed supplements.

However, several limitations should be noted. Dietary measurements are limited by methods of ascertainment, food composition databases and differences in bioavailability[292]. Genetic variability may play a large role in lycopene bioavailability. In one study, a combination of 28 single nucleotide polymorphisms (SNPs) on 16 genes accounted for 72% of lycopene bioavailability variation[59]. These differences in lycopene bioavailability may affect long-term blood lycopene status and consequently PCa risk[59,60]. Next, guidelines for PCa screening vary between countries, medical organizations and guideline groups[5]. One of the largest risk factor of PCa diagnosis, especially of non-aggressive disease, is screening. Differences in screening intervals and guidelines may affect PCa detection and incidence rates[6]. Additionally, the racial profiles of each study are different, which may contribute to differences in PCa risk. In the United States, African-Americans experience the highest incidence rates of PCa and have a 2.4-fold higher mortality rate compared to White Americans[4]. Likewise, Asian-Americans have the

lowest incidence rates of PCa and have a 50% lower mortality rate compared to White Americans[4].

This study further supports lycopene's protective role in prostate carcinogenesis and progression. In conclusion, our meta-analysis demonstrates an inverse association between dietary and circulating lycopene on PCa risk. Our data also indicate dose-responses between dietary and circulating lycopene and PCa incidence. Our results, along with those of other clinical and cohort studies, may suggest potential health benefits from improving lycopene status. In the dose-response for dietary lycopene, we found a 1% decreased PCa risk for each additional 2 mg of lycopene consumed. Tomatoes provide the American diet with more than 85% of dietary lycopene and contain many other potentially beneficial carotenoids and phytochemicals that may protect against PCa. Improving lycopene status is easy to achieve. According to the USDA National Nutrient Database, ¼ cup of tomato paste contains 19 mg of lycopene, one slice of watermelon contains 13 mg of lycopene, and 1 cup of cherry tomatoes provide 3.8 mg of lycopene. More high-quality research is required in order to elucidate the associations between lycopene and advanced PCa risk and to determine the underlying mechanisms behind its associations with PCa. Overall, our results support the hypothesis that consumption of lycopene-containing foods is inversely associated with PCa incidence.

Tables and Figures for Chapter 3

Table 3.1: Characteristics of included studies that evaluated lycopene intake.

Author, Year (Cohort)	Study Period	Country	Number of cases	Number of controls	Total no. participants in cohort	Average follow-up (yrs)	Age (SD)	Lycopene Intake (mg/day)	Adjustments	Study Type
Angalliu, 2011 (CSDLH)	1995-1998	Canada	661	1864	34,291	Cases 4.3, Subcohort 7.7	Cases: 66.2 (8.4), Subcohort: 69.3 (10.5)	Q1 Intake: 2.45 Q5 Intake: 15.87	Age, race, BMI, PA and Edu	Case Cohort
Antwi, 2016 (PCaP)	2004-2009	USA, North Carolina and Louisiana	370	1733	2,267	.25	40-79	T1 Intake: < 2.95 T3 Intake: 5.80-100.55	Age, race, PSA screenings, BMI, SS, Edu, income, NSAIDs, dietary fat, study site	Case-Control
Bosetti, 2004	1991-2002	Italy	1294	1451	2,745	—	Cases: 63 (46-74) Controls: 66 (46-74),	Lycopene intake not recorded	Age, study center, Edu, occupational PA at 30-39, BMI, FHPC	Case-Control
Cohen, 2000	1993-1996	USA, Seattle	628	602	1,230	—	40-64	Q1 Intake: < 4.9 Q4 Intake: > 9.9	Fat, energy, race, age, FHPC, BMI, PSA tests in last 5 yrs and Edu	Case-Control
Deneo-Pellegrini, 1999	1994-1997	Uruguay	175	233	408	—	40-89	Q1 Intake: < 1.30 Q4 Intake: > 3.30	Age, residence, Edu, FHPC, BMI, and total energy intake	Case-Control
Goodman, 2006 (MPC)	1994-1996	USA, North Carolina	77	174	251	—	Cases: 68 (64-71), Controls: 66 (59-72)	T1 Intake: < 0.73 T3 Intake: > 1.65	Age, race, BMI, fat, and total energy intake	Case-Control
Hodge, 2004	1994-1997	Australia	858	905	1,763	—	<70	Q1 Intake: <4.90 Q5 Intake: >11.09	State, age group, year, country of birth, SES, and FHPC	Case-Control
Jain, 1999	1989-1993	Canada	617	636	1,253	—	Cases: 69.8, Controls: 69.9	Q1 Intake: <2.10 Q4 Intake: >12.68	Age, log total energy, vas, SS, marital status, study area, BMI, Edu, MV, area of study, and nutrients	Case-Control

Table 3.1. (Continued)

Author, Year (Cohort)	Study Period	Country	Number of cases	Number of controls	Total no. of participants in cohort	Average follow-up (yrs)	Age (SD)	Lycopene Intake (mg/day)	Adjustments	Study Type
Jian, 2007	2001-2002	China	130	274	404	—	Cases: 72.7 (7.1), Controls: 71.4 (7.2)	Q1 Intake: <1.61 Q4 Intake: >4.92	Age, locality, Edu, income, marital status, FHPS, MET, BMI, fat intake, calories	Case-Control
Key, 1997	1989-1992	England	328	328	656	—	Cases: 68.1, Controls: 68.1	T1 Intake: <0.402 T3 Intake: >0.718	Social Class	Case-Control
Kirsh, 2006 (PLCO)	1993-2001	USA	1,254	—	29,361	4.2	55-74	Q1 Intake: 5.052 Q5 Intake: 17.59	Age, energy, race, study center, FHPC, BMI, SS, PA, Vit E, intake, diabetes, aspirin, PSA screens	Cohort
Kristal, 2010 (PCPT)	1994-2003	USA & Canada	1,703	7,856	9,559	7	Cases: 63.6 (5.6), Controls: 62.6 (5.4)	Q1 Intake: < 4.00 Q4 Intake: > 10.918	Age, race, FPHC, trt arm, and BMI	Nested Case-Control
Lewis, 2009	1998-2004	USA	373	382	860	—	Cases: 63.3 (8.2) Controls: 62 (10.7)	T1 Intake: < 2.22 T3 Intake: > 4.40	Edu, BMI, SS, FHPC in first degree relatives, alcohol intake	Case-Control
Lu, 2001	1993-1995	USA	65	132	197	—	Cases: 59.98 (6.2), Controls 41.9 (13.6)	Q1 Intake: < 1.46 Q4 Intake >3.45	Age, race, Edu, FHPC, alcohol, smoking pack-years and caloric intake	Case-Control
McCann, 2005 (WNYDS)	1986-1991	USA	443	538	971	—	Cases: 69.7 (6.9), Controls: 69.3 (7.3)	Q1 Intake: <3.90 Q4 Intake: >8.86	Age, BMI, cigarette SS, and total energy	Case-Control
Meyer, 1997	1990-1993	Canada	215	593	808	—	≥45	Intake not recorded	Age, Edu, FHPC, group, and energy	Case-Control
Norrish, 2000 (APS)	1996-1997	New Zealand	317	480	797	—	40-81	Q1 Intake: <0.66 Q4 Intake: >1.99	Height, age, total anti-inflammatory drugs, and SES	Case-Control

Table 3.1. (Continued)

Author, Year (Cohort)	Study Period	Country	Number of cases	Number of controls	Total no. of participants in cohort	Average follow-up (yrs)	Age (SD)	Lycopene Intake (mg/day)	Adjustments	Study Type
Park, 2015 (MEC)	1993-1996	USA	7,115	–	75,216	13.9	45-75	Q1 Intake: <1.62, Q5 Intake: >4.37	Age, race, FHPC, nutrients, alcohol, PA, diabetes, Edu, SS, height, BMI	Cohort
Parker, 1999	1986-1989	USA	81	–	1,177	Up to 9 years	40-86	T1 Intake < 0.34 T3 Intake > 0.55	Age, dietary factors including alcohol, SS, FHPC	Cohort
Sanderson, 2004	2000-2002	USA, South Carolina	407	393	800	–	65-79	Q1 Servings ≤ 2.6/week Q4 Servings ≥ 8.1/week	Age, race, location and FHPC	Case-Control
Schuurman, 2002 (NLCS)	1986-1992	Netherlands	642	1,525	58,279	6.3	Cases: 63.9, Controls: 61.4	Q1 Intake: 0.1 Q5 Intake: 2	Age, FHPC and SES	Case-Cohort
Shahar, 2011	2009	Malaysia	35	70	105	–	Cases: 67.6 (4.7) Controls: 67.8 (4.6)	Median: 2.5	Age, race, FHPC, and energy	Case-Control
Stram, 2006 (MEC)	1993-1996	USA	3,922	–	82,486	Up to 8 years	45-75	Q1 Intake: <0.75* Q5 Intake: >20.17*	Age, BMI, Edu, FHPC	Cohort
Wang, 2016 (CPS-II Nutrition Cohort)	1992-1993	USA	8,898	–	86,402	10.2	72 at diagnosis	Q1 Intake: <2.8 Q4 Intake: 6.1-30.2	Age, diag, race, year, PSA testing, Edu, trt, CVD, PA, SS and total dairy	Cohort
Zu, 2014 (HPFS)	1986-2010	USA	5,728	–	51,529	24	40-75	Q1 Intake: <3.69 Q5 Intake: 10.13-115.0	Age, height, BMI, FHPC, race, SS, PA, nutrients, total calories	Cohort

Abbreviations: years (yrs); standard deviation (SD); tertile (T); quartile/quintile (Q); body mass index (BMI); family history of prostate cancer (FHPC); prostate specific antigen (PSA); physical activity (PA); education (Edu); smoking status (SS); multivitamin (MV); socioeconomic status (SES); treatment (trt); cardiovascular disease (CVD); diagnosis (diag); Auckland Prostate Study (APS); Cancer Prevention Study II Nutrition Cohort (CPS-II Nutrition Cohort); Markers of Prostate Cancer Study (MPC); Canadian Study of Diet, Lifestyle, and Health Cohort (CSDLH); Health Professionals Follow-Up Study (HPFS); Multiethnic Cohort Study (MEC); Netherlands Cohort Study (NLCS); North Carolina-Louisiana Prostate Cancer Project (PCaP); Prostate Cancer Prevention Trial (PCPT); Prostate, Lung, Colorectal, and Ovarian Trial (PLCO); Western New York Diet Study (WNYDS) * signifies mg/1000 kcal/day

Table 3.2: Characteristics of included studies that evaluated circulating blood values of lycopene

Author, Year (Cohort)	Study Period	Country	Number of cases	Number of controls	Total no. of participants in cohort	Average follow-up (yrs)	Age (SD)	Circulating Lycopene (µg/dL)	Adjustments	Study Type
Beilby, 2010	1990-2004	Australia	96	225	6,493	—	Cases: 69.8 (7.2) Controls: 69.3 (6.7)	T1 Serum: ≤ 10.18 T3 Serum: 16.64-69.8	Age, vitamin A supplementation	Nested Case-Control
Chang, 2005	1996-1998	USA	118	52	170	—	Cases: 63.9 (7.0), Controls: 62.8 (6.6)	Median: 27.38	Age, SS, and height	Case-Control
Gann, 1999 (PHS)	1982-1995	USA	578	1,294	14,916	Up to 13 years	Cases: 60.7 Controls: 61.5	Q1 Plasma: ≤ 26.17 Q5 Plasma: ≥ 58.01	Age, follow up time at risk and SS	Nested Case-Control
Gill, 2009 (MEC)	1993-1996	USA	467	936	29,009	—	Cases: 88.9 (7.1), Control: 68.7 (7.1)	Q1 Plasma: 22.0 Q4 Plasma: 65.6	Fasting prior to blood draw, BMI, FHPC and Edu	Nested Case-Control
Goodman, 2003 (CARET)	1983-1996	USA	307	309	18,314	—	45-69	Q1 Serum: ≤ 22.9 Q4 Serum: ≥ 41.7	Age, Location, exposure population, sex, SS, time of randomization	Nested Case-Control
Hsing, 1990	1974-1986	USA	13	103	206	13	47-91, Cases 71	Cases Q1 Serum: ≤ 18 Q4 Serum: ≥ 42	Age, BMI, aspirin, marital status, ancestry, region, PA, vas, SS, alcohol and nutrients	Nested Case-Control
Huang, 2003 (CLUE I)	1974-1996	USA	182	364	546	17	Cases: 54 (9), Controls: 54 (9)	Q1 Serum: 21.7 Q5 Serum: 54.9	Total blood lipid levels, hours fasted, BMI and Edu	Nested Case-Control
Huang, 2003 (CLUE II)	1989-1996	USA	142	284	426	3.5	Cases: 66 (8), Controls: 66 (8)	Q1 Plasma: 24.3 Q5 Plasma: 62.8	Age, years since blood draw, disease stage at diag, SS, BMI at 21	Nested Case-Control

Table 3.2 (Continued)

Author, Year (Cohort)	Study Period	Country	Number of cases	Number of controls	Total no. of participants in cohort	Average follow-up (yrs)	Age (SD)	Circulating Lycopene (µg/dL)	Adjustments	Study Type
Karppi, 2009 (KIHD)	1984-1989	Finland	55	—	997	12.6	42-60	T1 Serum: <4.30 T3 Serum: >10.2	Age, exam year, alcohol, FHPC, PA, waist-to hip-ratio, Edu, SS, and serum folate	Cohort
Key, 2007 (EPIC)	1999-2003	Europe	966	1064	137,001	4	Cases: 60.4 (5.8) Controls: 60.1 (5.7)	Q1 Plasma: <15.04 Q5 Plasma: >49.37	BMI, SS, alc intake, PA, marital status, Edu	Nested Case-Control
Kristal, 2011 (PCPT)	1994-2003	USA & Canada	1,683	1,752	3,435	7	Cases: 63.7 (5.6) Controls: 63.6 (5.6)	Q1 Serum: <26.3 Q4 Serum: >46.6	Age, race, diabetes, serum cholesterol, BMI	Nested Case-Control
Lu, 2001	1993-1995	USA	65	130	195	—	Cases 59.98 (6.2), Controls 41.9 (13.6)	Q1 Plasma: <9.59 Q4 Plasma: >21.53	Race, age, Edu, FHPC, pack-years of smoking, alc, caloric intake	Case-Control
Nomura, 1997	1971-1975	USA, Hawaii	142	142	6,860	18-22	Cases 62 (52-74), Controls 62 (52-74)	Median Serum: 13.4	None	Nested Case-Control
Nordström, 2016	2000-2007	USA, San Francisco	138	421	559	0.3	59 (6.9)	Q1 Plasma: 18.3 Q4 Plasma: 61.4	age, cholesterol, smoking at diag, and race	Cohort
Peters, 2007 (PLCO Trial)	1993-2001	USA	692	841	28,243	8	55-74	Q1 Plasma: 30.5 Q5 Plasma: 108.4	Age, time since initial screening, year of blood draw, study center	Nested Case -Control
Vogt, 2002	1986-1989	USA, Atlanta	228	209	437	3	40-79	Q1 Serum: 0.5-10.7 Q4 Serum: 24.8-57.4	Age, race, study center, month	Case-Control

Table 3.2 (Continued)

Author, Year (Cohort)	Study Period	Country	Number of cases	Number of controls	Total no. of participants in cohort	Average follow-up (yrs)	Age (SD)	Circulating Lycopene (µg/dL)	Adjustments	Study Type
Wu, 2004 (HPFS)	1986-1995	USA	450	450	51,529	9	40-75	Plasma lycopene not recorded	Cholesterol, supp, FHPC, BMI, height, exercise, vas, SS	Nested Case-Control
Zhang, 2007	1988-2003	USA, Arkansas	193	197	390	5	Cases 64.4 (9), Controls 59.4 (10.5)	Q1 Plasma: 14.05 Q4 Plasma: 51.37	Age, race, BMI, Edu, SS and other carotenoids	Case-Control

Abbreviations: years (yrs); standard deviation (SD); tertile (T); quartile/quintile (Q); body mass index (BMI); family history of prostate cancer (FHPC); vasectomy (vas); physical activity (PA); education (Edu); smoking status (SS); alcohol (alc); supplement (supp); treatment (trt); diagnosis (diag); Carotene and Retinol Efficacy Trial (CARET); Give us a CLUE to Cancer Study I (CLUE 1); Give us a CLUE to Cancer Study II (CLUE II); European Prospective Investigation into Cancer Study (EPIC); Health Professionals Follow-Up Study (HPFS); Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD); Multiethnic Cohort Study (MEC); Prostate, Lung, Colorectal, and Ovarian Cancer Trial (PCPT); Physicians' Health Study (PHS); Prostate, Lung, Colorectal, and Ovarian Cancer Trial (PLCO)

Table 3.3. Quality assessment of all studies investigating lycopene

Source	Selection				Comparability[5]	Exposure			Total[9]
Author, Year	Definition[1]	Representative	Selection	Definition[4]		Ascertainment[6]	Method[7]	Rate[8]	
Angalliu, 2011	★	★	★	★	★★	★	★	★	9
Antwi, 2016	★	★	★	0	★★	★	★	★	8
Beilby, 2010	★	★	★	★	★★	★	★	★	9
Bosetti, 2004	★	★	★	★	★★	★	★	★	9
Chang, 2005	★	★	★	★	★★	★	★	★	9
Cohen, 2000	★	★	★	★	★★	★	★	★	9
Deneo-Pellegrini, 199	★	★	0	★	★★	★	0	★	7
Gann, 1999	★	★	★	★	★★	★	★	★	9
Gill, 2009	★	★	★	★	★	★	★	★	8
Goodman, 2003	★	★	★	★	★★	★	★	★	9
Goodman, 2006	★	★	★	★	★★	0	★	★	8
Hodge, 2004	★	★	★	★	★★	0	★	★	8
Hsing, 1990	★	★	★	★	★★	★	★	★	9
Huang, 2003 (CLUE 1	★	★	★	★	★	★	★	★	8
Huang, 2003 (CLUE 2	★	★	★	★	★★	★	★	★	9
Jain, 1999	★	★	★	★	★★	0	★	★	8
Jian, 2007	★	★	★	★	★★	★	★	★	9
Karppi, 2009	★	★	★	★	★★	★	★	★	9
Key,1997	★	★	★	★	★	★	★	★	8
Key, 2007	★	★	★	★	★	0	★	★	7
Kirsh, 2006	★	★	★	★	★★	★	★	★	9
Kristal, 2010	★	★	★	★	★★	★	★	★	9
Kristal, 2011	★	★	★	★	★★	★	★	★	9
Lewis, 2009	★	★	★	★	★	★	★	★	8
Lu, 2001	★	★	★	★	★★	★	★	★	9
McCann, 2005	★	★	★	★	★★	0	0	0	6
Meyer, 1997	★	★	★	★	★★	★	★	★	9
Nomura, 1997	★	★	★	★	0	★	★	★	7
Nordstrom, 2016	★	★	★	0	★★	★	★	★	8
Norrish, 2000	★	★	★	★	★★	★	★	★	9
Park, 2015	★	★	★	★	★★	★	★	★	9
Parker, 1999	★	★	★	★	★★	0	0	★	7
Peters, 2007	★	★	★	★	★★	★	★	★	9
Sanderson, 2004	★	★	★	★	★★	★	0	★	8
Schuurman, 2002	★	★	★	★	★★	★	★	★	9
Shahar, 2011	★	★	★	★	★★	★	★	★	9
Stram, 2006	★	★	★	★	★★	★	★	★	9
Vogt, 2002	★	★	★	★	★★	0	★	★	8
Wang, 2016	★	★	★	★	★★	★	★	★	9
Wu, 2004	★	★	★	★	★	★	★	★	8
Zhang, 2007	★	★	★	★	★★	0	★	★	8
Zu, 2014	★	★	★	★	★★	★	★	★	9

1 indicates that cases are independently validated (0,1star); 2, cases have prostate cancer, representative of population (0,1); 3, controls are selected from same population as cases (0,1); 4, controls have no history of prostate cancer (endpoint) (0,1); 5, study controls for most important factor (age) and other factors (0-2); 6, same method of ascertainment for cases and controls (0,1); 7, validated and comparable method for lycopene determination (0,1), 8, unbiased response between groups (0,1); 9, total: minimum equals 1; maximum equals 9 stars

Table 3.4. Subgroup analysis between lycopene and prostate cancer risk

	Dietary Intake of Lycopene				Circulating Values of Lycopene			
	No. of studies	Relative Risk (95% CI)	I^2 (%)	P-value	No. of studies	Relative Risk (95% CI)	I^2 (%)	P-value
Overall model	21	0.88 (0.78-0.98)†	55.8	**0.017**	17	0.88 (0.79-0.98)	26.2	**0.019**
Study type								
Cohort/case cohort	6	0.93 (0.87-0.99)	11.0	**0.030**	1	0.78 (0.37-1.65)	0.0	0.516
Case-control	15	0.82 (0.68-0.999)†	63.7	**0.049**	4	0.60 (0.32-1.13)†	59.8	0.115
Nested case-control	0	–	–	0.657	12	0.91 (0.81-1.02)	3.6	0.093
Continent								
North America	12	0.93 (0.87-0.99)	47.0	**0.036**	14	0.87 (0.78-0.98)	38.2	**0.025**
Europe	4	0.93 (0.79-1.10)	0.0	0.389	2	0.94 (0.70-1.26)	0.0	0.670
Other	5	0.59 (0.35-0.99)†	77.3	**0.046**	1	0.77 (0.40-1.48)	–	0.431
Adjustments								
High Quality	17	0.89 (0.79-1.00)†	53.7	0.051	15	0.86 (0.77-0.97)	32.9	**0.013**
Mid Quality	4	0.81 (0.64-1.01)	48.4	0.058	2	0.99 (0.74-1.33)	0	0.946
Adjusts for age	18	0.85 (0.76-0.96)†	58.2	**0.007**	10	0.91 (0.80-1.04)	40.2	0.169
No adj for age	3	1.12 (0.85-1.47)	7.8	0.405	7	0.81 (0.67-0.99)	0.0	**0.034**
Adjusts for BMI	12	0.89 (0.77-1.02)†	66.4	0.099	9	0.85 (0.75-0.97)	28.2	**0.013**
No adj for BMI	9	0.84 (0.73-0.099)	11.5	**0.033**	8	0.96 (0.78-1.17)	27.2	0.656
Adjusts for smoking	8	0.93 (0.87-1.00)	46.8	**0.046**	10	0.80 (0.66-0.97)	35.9	**0.022**
No adj for smoking	13	0.81 (0.67-0.98)†	60.1	**0.032**	7	0.92 (0.80-1.05)	4.6	0.215
Adjusts for FHPC	14	0.94 (0.88-0.996)	30.7	**0.038**	4	0.66 (0.49-0.88)	41.8	**0.005**
No adj for FHPC	7	0.69 (0.51-0.93)†	72.6	**0.015**	13	0.92 (0.82-1.04)	1.7	0.163
Adjusts for education	10	0.95 (0.77 -1.17)†	67.9	0.627	7	0.80 (0.66-0.97)	23.1	**0.025**
No adj for education	11	0.88 (0.82-0.94)	29.3	**<0.001**	10	0.92 (0.82-1.04)	28.4	0.187
Adjusts for PA	5	0.93 (0.87-0.998)	0.0	**0.043**	5	0.78 (0.63-0.95)	20.8	**0.016**
No adj for PA	16	0.81 (0.67-0.98)†	63.0	**0.030**	12	0.92 (0.81-1.05)	25.3	0.213
Adjusts for alcohol	4	0.99 (0.72-1.36)†	52.4	0.930	5	0.80 (0.64-0.99)	39.9	**0.041**
No adj for alcohol	17	0.85 (0.75-0.96)†	54.9	**0.008**	12	0.91 (0.80-1.03)	21.5	0.132
Adjusts for energy	10	0.82 (0.66-1.00)†	72.2	0.051	0	–	–	–
No adj energy	11	0.94 (0.85-1.03)	19.8	0.186	17	0.88 (0.79-0.98)	26.2	**0.019**

Figure 3.1 Literature search and study selection flow chart.

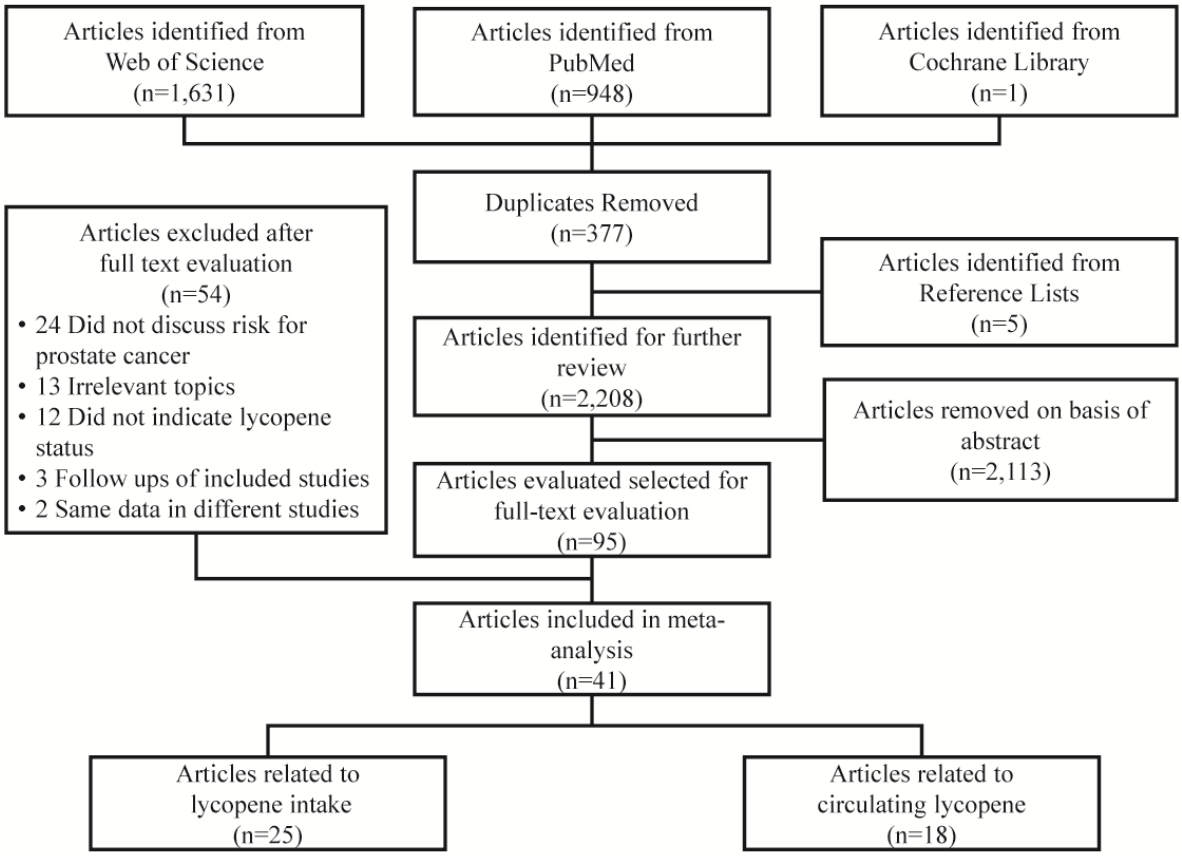
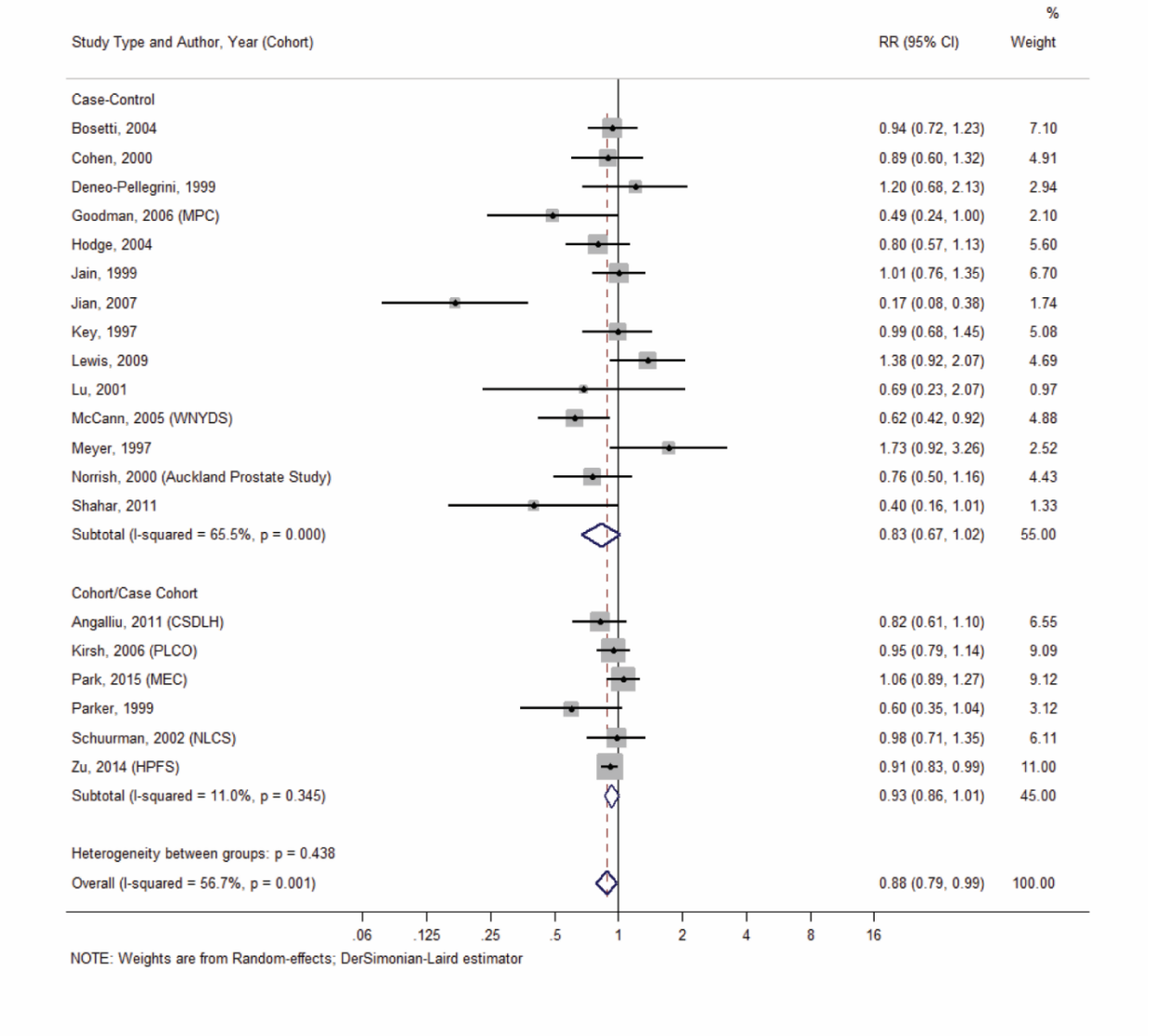
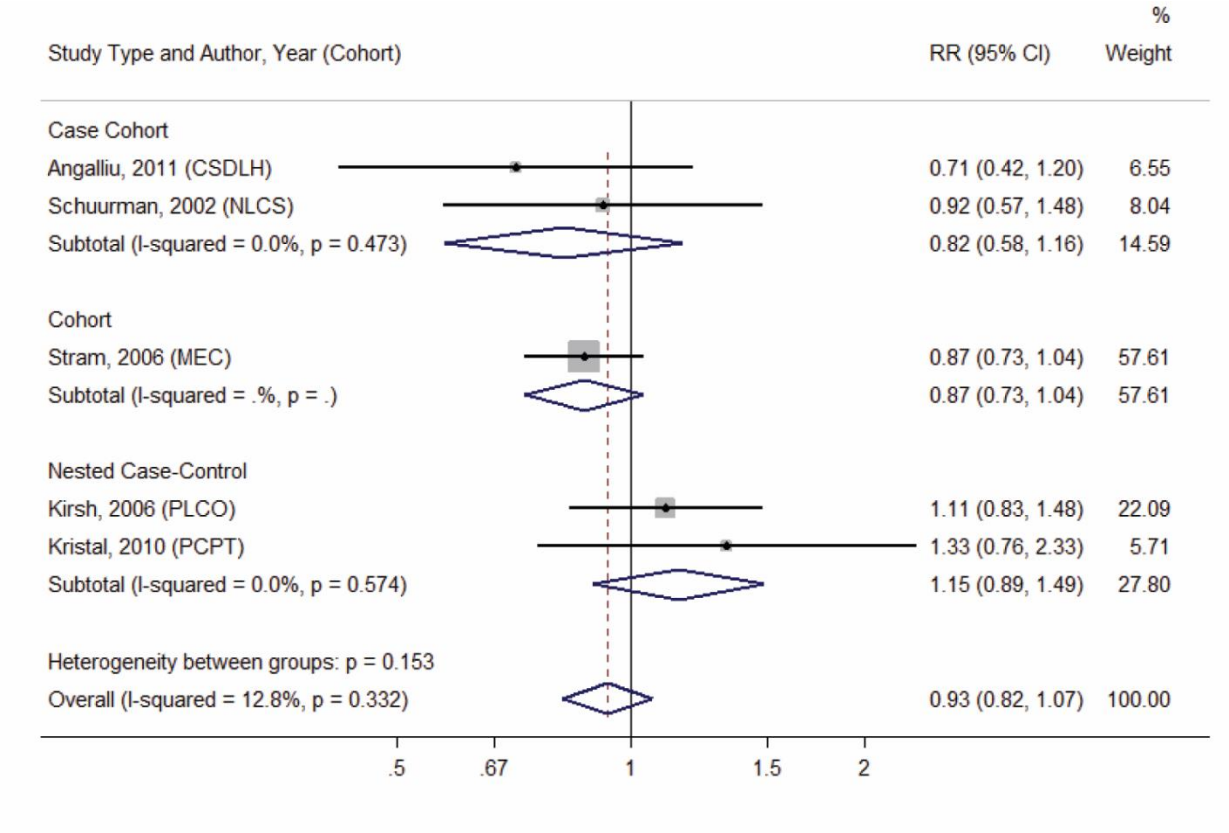


Figure 3.2 Forest plot for association of highest vs lowest intake of lycopene on PCa risk.



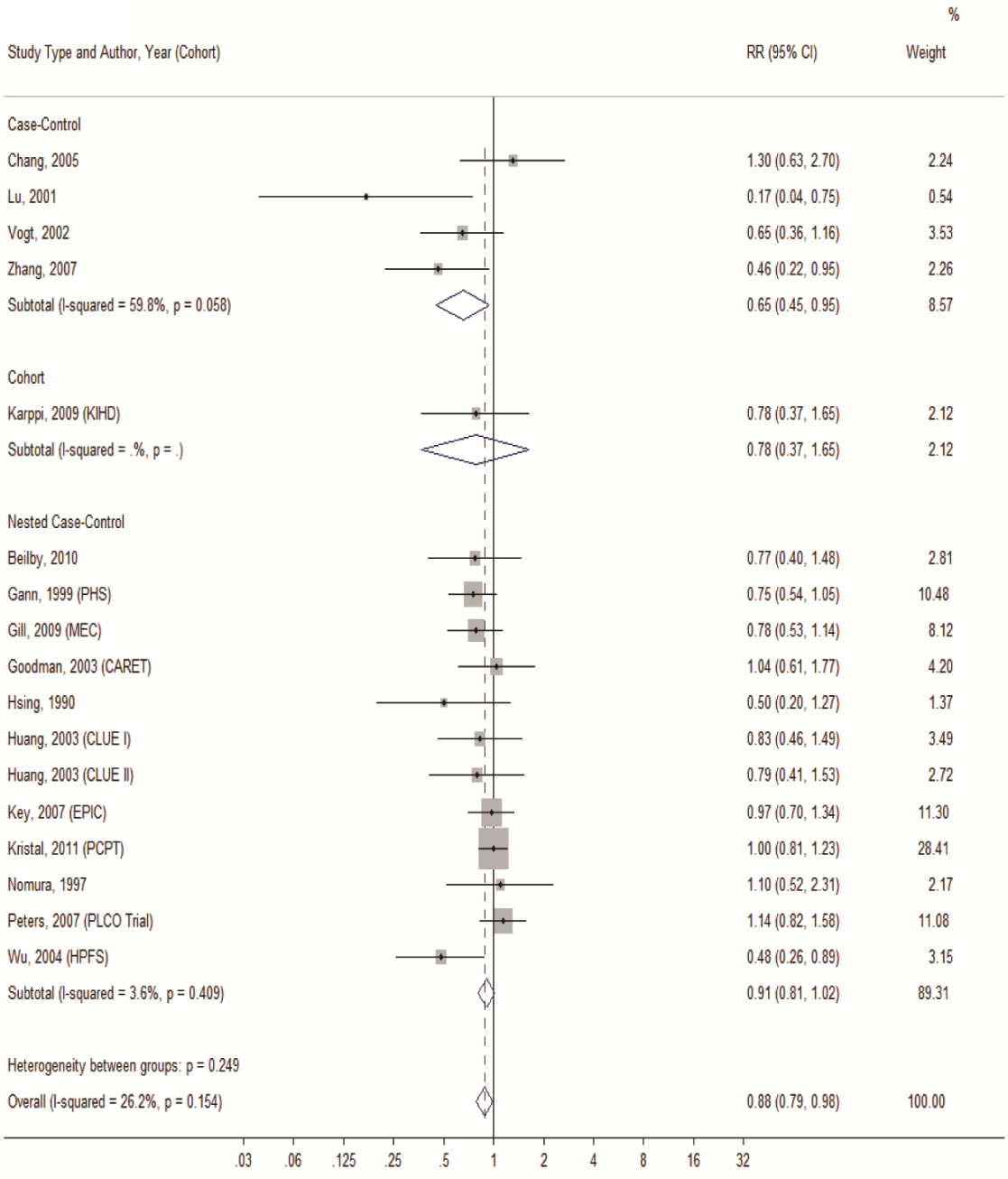
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 3.3 Forest plot for association of highest vs. lowest intake of lycopene on advanced PCa risk**.**



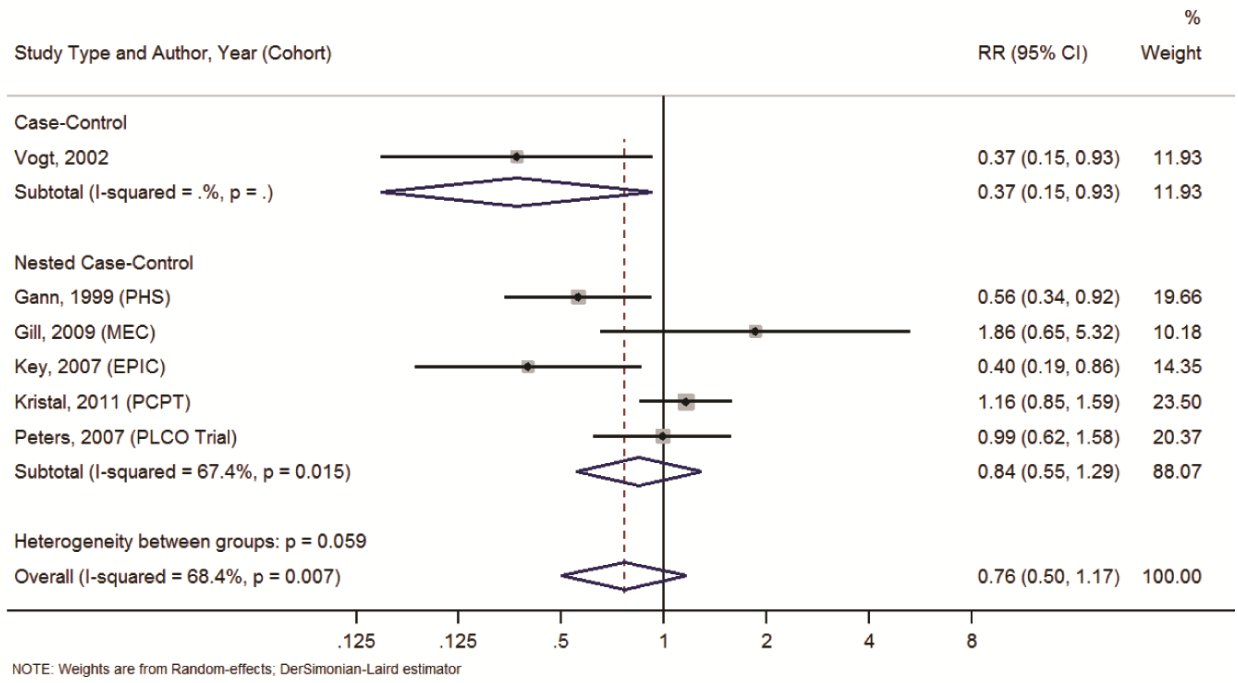
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 3.4 Forest plot for association of highest vs lowest circulating lycopene on PCa risk.



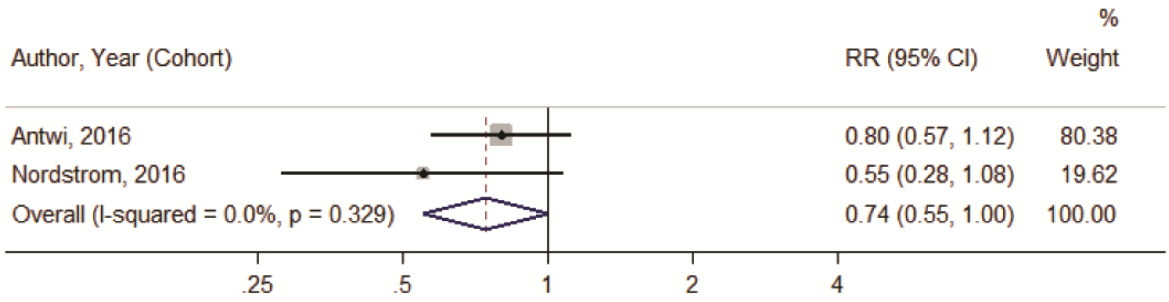
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 3.5 Forest plot for the association of highest vs. lowest circulating lycopene on advanced PCa risk.



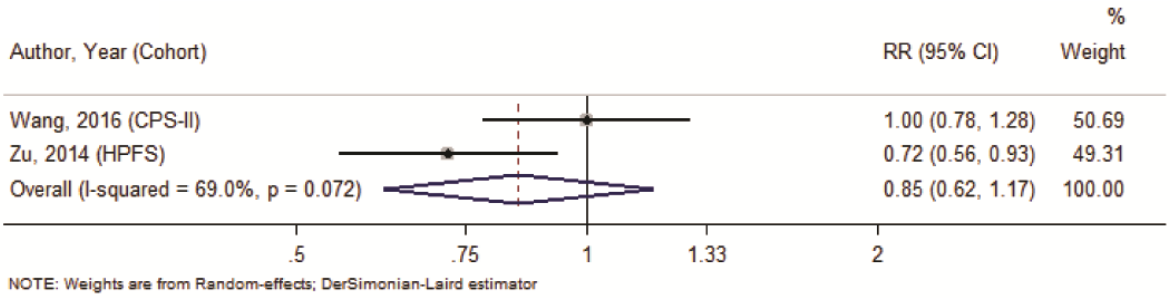
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 3.6 Forest plot for the association of highest vs lowest lycopene concentrations on PCa aggressiveness.



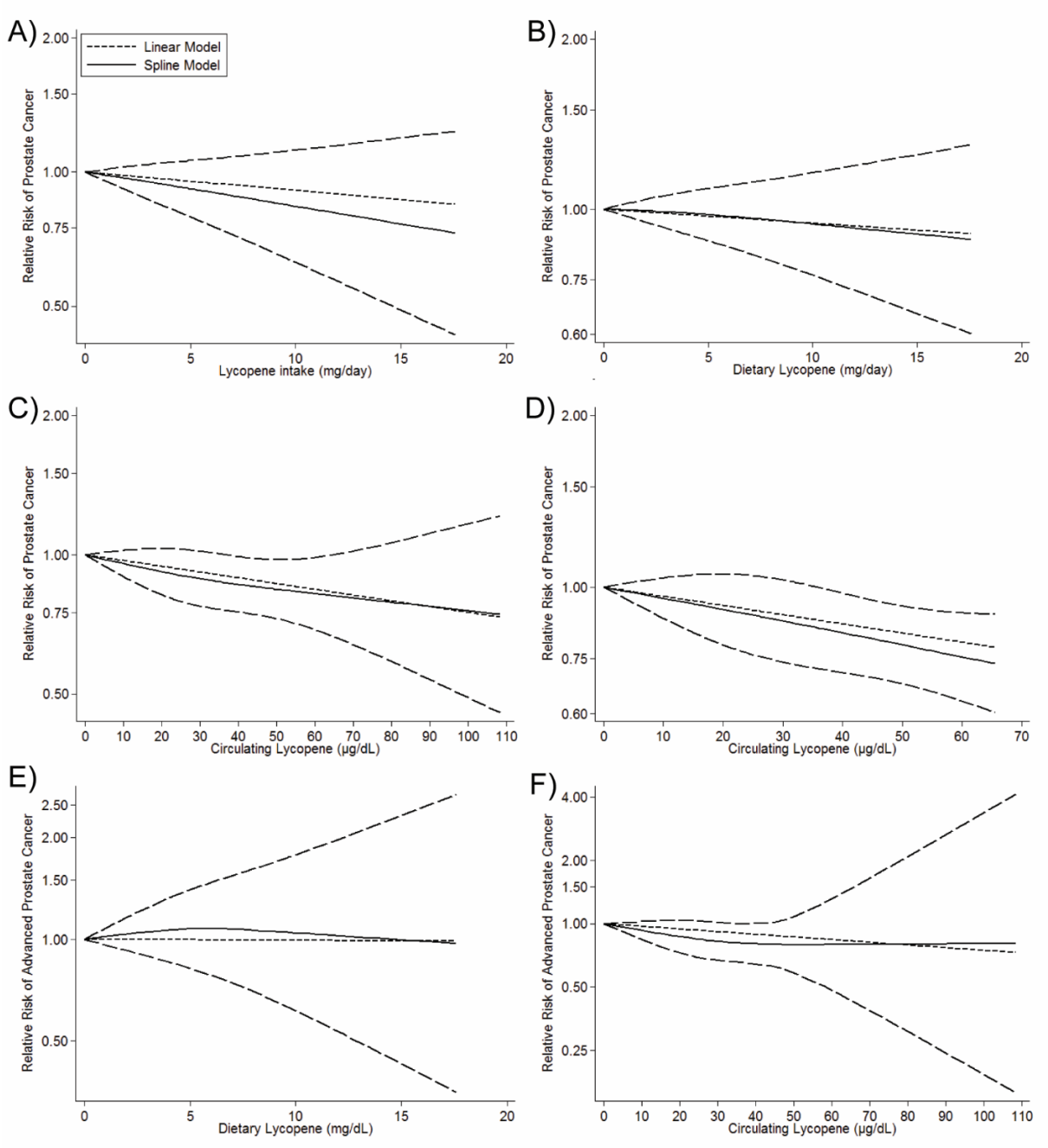
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 3.7 Forest plot for the association of highest vs lowest lycopene concentrations on PCa mortality.



This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure 3.8 Dose-response analysis of lycopene and PCa risk.



The solid black line is the nonlinear model curve for published relative risk and the two dotted lines are the corresponding 95% confidence intervals. The short-dashed line is the linear model curve for the published relative risks (RR); (A) dietary lycopene (mg/day) and risk of PCa; (B) dietary lycopene (mg/day) and risk of PCa after exclusion of one study (C) circulating lycopene concentrations (µg/dL) and risk of PCa; (D) circulating lycopene concentrations (µg/dL) and risk of PCa after exclusion of one study E) dietary lycopene (mg/day) and risk of advanced PCa (F) circulating lycopene concentrations (µg/dL) and risk of advanced PCa.

Chapter 4: Tomatoes or lycopene do not alter castration resistant prostate cancer progression in the TRAMP model

[c,d] *Abstract*

Background: Dietary tomato products or lycopene protect against prostate carcinogenesis, but their ability to reduce the emergence of castration-resistant prostate cancer (CRPC) is unknown. We hypothesized that tomato or lycopene products would reduce the emergence and growth of CRPC.

Methods: Transgenic adenocarcinoma of the mouse prostate (TRAMP) mice were castrated at 12-13 weeks of age and the emergence of CRPC was monitored by ultrasound in each study. In Study 1, TRAMP mice (n=80) were weaned onto an AIN-93G-based control diet (Con-L, n=28), a 10% tomato powder diet (TP-L, 10% lyophilized w/w, n=26), or a control diet followed by a tomato powder diet after castration (TP-Int1, n=26). In Study 2, TRAMP mice (n=85) were randomized onto a control diet with placebo beadlets (Con-Int, n=29), a tomato diet with placebo beadlets (TP-Int2, n=29) or a control diet with lycopene beadlets (Lyc-Int, n=27) following castration (12 weeks of age). Tumor incidence and growth were monitored by ultrasound beginning at 10 weeks of age. Mice were euthanized 4 weeks after tumor detection or at 30 weeks of age if no tumor was

[c] Joe L. Rowles primarily contributed to Study 2 and assisted with Study 1. Study 1 was primarily conducted by Joshua W. Smith. For congruency, both studies are reported together as Chapter 4.

[d] The content of this chapter has been accepted by Journal of Nutrition (Rowles J.L. III, Smith J.W., Applegate C.C., Miller R.J, Wallig M.A., Kaur A., Sarol Jr J.N., Musaad S., Clinton S.K. O'Brien Jr. W.D, Erdman Jr, J.W. Journal of Nutrition, 2020 DOI: 10.1093/jn/nxaa107)

detected. Tissue weights were compared by ANOVA followed by Dunnett's test. Tumor volumes were compared using generalized linear mixed model regression.

Results: Ultrasound estimates for the *in vivo* tumor volume were strongly correlated with tumor weight at necropsy (R^2 =0.75 and 0.94, p<0.001 for both Study 1 and 2, respectively). Dietary treatments after castration did not reduce cancer incidence, time to tumor detection, or final tumor weight.

Conclusions: In contrast to studies of *de novo* carcinogenesis in multiple preclinical models, tomato components did not reduce the emergence of CRPC in the TRAMP model. It is possible that specific mutant subclones of prostate cancer may continue to show some anti-proliferative response to tomato components, but further studies are needed to confirm this.

Introduction

Prostate cancer (PCa) is the second leading cause of cancer-related deaths among men in the United States.[293] Androgen deprivation therapy (ADT) has been the primary therapy for advanced and metastatic PCa for over 70 years.[294] Historically, ADT was performed by surgical castration following the discovery that testosterone was critical to prostate growth and function in laboratory models.[33,295] In recent decades ADT increasingly has been accomplished by pharmacologic agents and integrated into effective multimodality treatment plans for locally advanced and high-grade localized prostate cancer, and in salvage regimens for local recurrence following prostatectomy.[2, 5] This has led to an improvement in quality of life, sexual function, and life

expectancy.[294,296] Unfortunately, ADT alone is rarely curative as genetic instability within the cancer cells lead to the emergence of mutant sub-clones that progress in spite of castrate serum concentrations of testosterone.[36] This late and often lethal phenotype is termed castration-resistant prostate cancer (CRPC).[36]

Following PCa diagnosis, patients often seek information about food and supplements that may improve their response to therapies, quality of life, and survival. Tomatoes and lycopene are two of the most frequently mentioned foods or supplements by social media, lay press, and purveyors of alternative therapy as having a protective effect on prostate cancer activity. Consumption of tomatoes or their predominant carotenoid, lycopene, has been associated with lower PCa risk in many epidemiological studies.[127,132] Interestingly, increased tomato or lycopene consumption in epidemiological cohorts appear to have a greater reductions in risk with lethal or aggressive PCa.[94,95,285] In agreement with the human epidemiological evidence, studies in multiple rodent models support the hypothesis that dietary tomato or lycopene reduce *de novo* prostate carcinogenesis.[114,116,297] However, the potential efficacy of dietary tomato or lycopene as a component of an integrated treatment plan to reduce the progression of CRPC has not been thoroughly investigated in experimental systems.

Based on the epidemiological and preclinical evidence for PCa incidence, many men with PCa undergoing ADT or with CRPC might choose to consume lycopene supplements without evidence from definitive phase III human trials. Although some groups have explored the activity of tomato carotenoids on the growth of androgen-insensitive PCa xenografts,[298-300] these short-term studies in models of tumorigenesis do not recapitulate the complex and multiple pathways involved in the malignant transition

from androgen sensitive to the castration-resistant state. Additionally, the use of pharmacological doses of lycopene, far beyond what is relevant to the diet, is a concern because little is known regarding the risks of such intake in humans.[301,302] Although these data suggest that dietary tomato or lycopene may provide a benefit to men with advanced androgen-sensitive PCa, a lack of preclinical data on which to base more definitive trials remains a gap in the literature.

We sought to address this hypothesis by investigating whether lifelong tomato consumption, a later dietary tomato intervention, or a later dietary lycopene intervention would be effective in reducing the emergence and growth of CRPC tumors in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. To investigate this hypothesis, we conducted 2 studies. Study 1 investigated the potential for lifelong or post-castration tomato interventions to reduce CRPC incidence and progression in castrated TRAMP mice. Study 2 evaluated the potential for post-castration tomato or lycopene interventions to reduce CRPC incidence and progression in castrated TRAMP mice. To our knowledge, this is the first report to evaluate the efficacy of dietary tomato or lycopene combined with castration (as a model of ADT) to reduce the incidence and progression of CRPC in a rodent model.

Materials and Methods

Diets

Tomato paste (Contadina®, San Francisco, CA) was purchased from a local supermarket in September 2014 and July 2015 for Study 1 and in April 2016 (Study 2); followed by lyophilization in a VirTis Freezemobile 12SL/Unitop 600 SL freeze dryer (SP

Scientific, Warminster, PA). Dried yield was ~25% of wet mass. Lyophilized tomato paste (TP) was ground to fine powder in a tabletop food processor, transferred to resealable gallon bags (air removed), and kept in the dark at -20˚C until diet mixing.

Two experimental diets were used in Study 1: a powdered, AIN-93G-based control diet and the same diet modified to contain 10% (w/w) lyophilized tomato paste (TP). In Study 2, similar control and tomato diets were used with the addition of placebo beadlets (0.47 g/ kg diet, DSM, Netherlands). Study 2 also included a powdered AIN-93G-based control diet containing lycopene beadlets (Lyc) (0.47g of 10% lycopene beadlets/ kg diet, DSM, Netherlands). The composition of each diet is described in Table 4.1. Ingredients were mixed using a commercial mixer (Hobart, Illinois. USA). Proximate analysis was performed on the 100% tomato paste powder and diet formulas were balanced for total energy, carbohydrates, protein, fat, fiber, and moisture. New diets were formulated every 1.5-2 months. Seven (Study 1) or six (Study 2) batches of the 10% tomato diet were made throughout the course of the study and each was analyzed for carotenoid content by high-performance liquid chromatography (HPLC).

Mouse breeding, genotyping, and housing

The University of Illinois Laboratory Institutional Animal Care and Use Committee reviewed and approved all experimental procedures (Study 1 protocol number 14296; Study 2 protocol number 16078). Male C57BL/6-Tg(TRAMP)8247Ng/J (C57BL/6 TRAMP$^{+/-}$), female C57BL/6J, and female FVB/NJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). A breeding colony was maintained with crosses of C57BL/6J females and C57BL/6 TRAMP$^{+/-}$ males. Male F_1 offspring of FVB/NJ females and C57BL/6 TRAMP$^{+/-}$ males were used for the study. Tail DNA of pups was isolated

with Extract-N-Amp™ Tissue PCR kits (Sigma-Aldrich, St. Louis, MO) and mice were genotyped to confirm transgene presence. Males carrying the probasin:SV40-Tag transgene (hereafter referred to as TRAMP mice) were weaned at 3 weeks of age and enrolled into the study via rolling admission. Mice were housed under controlled conditions (12-hour light/dark cycle, 22˚C, 55% humidity), weighed weekly, and diet was added 3 times per week.

Study 1. Allotment of mice

TRAMP mice were acclimated to the AIN-93G control diet from weaning at 3 to 4 weeks of age and randomized to consume control diet (Con, n=54) or 10% TP (TP-L, n=26). Following castration between 12-14 weeks of age, mice were switched from the control diet to an intervention of 10% TP (TP-Int1, n=26) or remained on the control diet (Con-L, n=28).

Study 2. Allotment of mice

TRAMP mice were acclimated to the AIN-93G control diet from weaning at 3 weeks of age until castration at 12 weeks of age. Following castration, TRAMP mice consumed dietary treatments of control diet with placebo beadlets (Con-Int, n=29), an AIN-93G diet modified to contain 10% lyophilized tomato paste with placebo beadlets (TP-Int2, n=29), or the control diet with lycopene beadlets (Lyc-Int, n=27).

Castration Surgery

Mice were surgical castrated between 12 and 14 weeks of age under inhalation isoflurane for general anesthesia on a heated platform. The mean age at castration was 13.13 ± 0.06 weeks in Study 1 and 12.10 ± 0.03 weeks in Study 2. At this age, TRAMP

mice exhibit nearly 100% incidence of high-grade prostatic intraepithelial neoplasia or microscopic well- to moderately-differentiated adenocarcinoma.[88,135] Subcutaneous injections of an analgesic (buprenorphine, 0.05 mg/kg or carprofen, 5 mg/kg) was given pre- and post-surgery. Figure 4.1 displays the study designs with diet interventions, castration, and necropsy for both studies.

In vivo ultrasound tumor screening and measurement

Beginning at 10 weeks of age, biweekly (every 2 weeks) *in vivo* ultrasound imaging was used for longitudinal screening and tumor volume measurement. Inhalation isoflurane was used for general anesthesia. Ultrasonic scans were obtained through the ventral body wall while in dorsal recumbency on a heated table using the Vevo 2100 pre-clinical ultrasonic imaging platform (VisualSonics, Inc., Toronto, Canada). Scans were conducted in 3D B-mode, and frames were collected in a caudal to cranial direction at intervals of approximately 0.152 mm. Serial 2D image slices were used to generate prostatic or tumor volume estimates as previously described.[303] Mice with prostate tumors identified at 14 weeks of age or later were switched from biweekly ultrasound screening to weekly ultrasound scans in order to measure CRPC tumor volume.

Necropsy

Mice were euthanized for necropsy based upon the following criteria: (a) a moribund clinical status, (b) a four-week time period after a tumor mass was detected by ultrasound, (c) a tumor volume exceeding 5000 mm^3, or (d) 30 weeks of age with no tumor detected by ultrasound. Study 1 mice were euthanized by CO_2 asphyxiation under isoflurane-induced general anesthesia, followed by cervical dislocation. Study 2 mice

were exsanguinated by cardiac puncture under deep anesthesia followed by cervical dislocation. When possible, the prostate was dissected into individual lobes (anterior, dorsal, lateral and ventral). Suspected malignant prostate masses (tumors) were dissected from the remaining prostate. Individual prostate lobes, malignant prostate tumors, seminal vesicles, liver, lungs, and epididymal adipose tissue were weighed and snap frozen in liquid N_2 and stored at -80˚C for future analysis. Gross metastases to the lungs, liver, kidneys, urethra, and regional lymph nodes (medial iliac and lumbar aortic, when present) were identified by visual inspection, and tissues were fixed in 10% neutral-buffered formalin for 12 to 24 hours and held in 70% aqueous ethanol until paraffin embedding.

Histopathology and immunohistochemistry

Tissues were embedded in paraffin and 4-µm-thick sections were stained with hematoxylin and eosin (H&E). A blinded examiner (SKC or MAW) evaluated the extent and severity of neoplasia in prostate and tumor sections as previously described.[304] Metastases were confirmed by H&E and SV-40 staining, and the emergence of poorly differentiated cancer exhibiting a stereotypic neuroendocrine phenotype was determined by staining against synaptophysin (ABCAM, Cambridge, MA).

Carotenoid measurement

Diet and tissue carotenoids were extracted and analyzed by HPLC as previously described.[62,305] Approximately 25 mg diet, 300 mg tumor tissue, 200 µL serum, and 100 mg liver tissue were used for analysis. Carotenoids in the serum were analyzed by HPLC-tandem mass spectrometry as previously described[306] in Study 1 and by HPLC in Study

2.[62,305] Due to castration, anterior prostates atrophied and were too small for individual assay. Thus, anterior prostates from 8-12 mice (one lobe per mouse) were pooled to achieve quantifiable signal. Anterior prostates from 8 and 12 mice (one lobe per mouse) in Study 1 were pooled into two individual replicates per treatment. Anterior prostates from 2-5 animals (one lobe per mouse) were pooled in Study 2.

Statistical analysis

Parallel statistical analyses were conducted for both studies. SAS (version 9.4, SAS Institute, Cary, NC) was used for statistical analyses. In total, 80 mice from Study 1 (Con-L, n = 26; TP-L, n = 28; TP-Int1, n = 26) and 85 mice from Study 2 (Con-Int, n=29; TP-Int2, n=29; Lyc-Int, n=27) were included in the final analysis. Descriptive statistics of mouse characteristics such as enrollment age, age at castration, age at euthanasia, weight at euthanasia, occurrence and sites of lesions were obtained using means and standard error for quantitative variables and frequencies and percentages for dichotomous variables. Carotenoid accumulation was compared between carotenoid-containing treatments by t-test. Body weight at necropsy and organ weights were assessed by ANOVA with multiple comparisons correction by Dunnett's test. Cancer incidence was assessed by Fisher's exact test between the control group and each treatment group. Survival curves were generated using product-limit estimation, with time from castration to appearance of ultrasound-detected tumor treated as the duration of tumor-free survival. We tested for significance of the differences in the survival rates between treatments by employing the log-rank test with PROC LIFETEST. To control for age and weight as covariates, proportional hazards regression using PROC PHREG was performed.

In both studies, 56 mice developed lesions that were detectable by ultrasound with weekly tumor volume measurements. Due to the rapid rate of weekly volume increase and heterogeneity of data, tumor volumes were transformed by natural logarithms. Generalized linear mixed model regression using PROC GLIMMIX was employed to examine differences between the treatment groups for rate of tumor growth during the five consecutive weeks of tumor volume monitoring. The model was fit to the data assuming a lognormal distribution of the outcome. A random intercept and slope, treatment effects as well as the interaction of treatment and time were included in the model. The latter term represented the differences in the rate of tumor growth (slopes) between treatment groups. Experimental units (mice) were nested within treatment group. Age and weight at castration were included in the model as covariates. Pairwise comparisons across treatment groups were conducted using the LSMESTIMATE statement and the p-values were adjusted for multiple comparisons using the Sidak test.[307] We performed sensitivity analysis in the regression using generalized linear mixed models in two ways: 1) by considering a weighted least squares estimation of the parameter models, and 2) by removing influential observations based on studentized residuals. Differences in the tumor weight at euthanasia, week 0 tumor volume, week 4 tumor volume, and the final non-missing tumor volume among the treatment groups were evaluated through generalized linear regression. Generalized linear regression was conducted using PROC GLMSELECT where age and weight at castration were considered as potential confounders. Sensitivity analyses were conducted for both studies by using a weighted least squares approach and by removing extreme observations. Finally, metastases were not statistically assessed due to insufficient power

to evaluate these endpoints. The primary outcomes for this study were cancer incidence, time to tumor detection, tumor growth rate, final tumor volume, and tumor weight. Unless otherwise stated, $p<0.05$ was considered significant. All values are reported as mean ± standard error of mean (SEM).

Results

Carotenoid content of diet and accumulation in tissues

The carotenoid composition of the tomato and lycopene diets are shown in Figure 4.2. Lycopene was the predominant carotenoid (~40 nmol/g, ~50% of total carotenoids) in the TP diets for both studies. Within the Lyc-Int diet, lycopene content was 139 nmol lycopene/g diet. No significant differences in tissue carotenoid accumulation were observed between tomato treatments in Study 1 (Table 4.2). Similarly, no significant differences in tissue lycopene accumulation were observed between Lyc-Int and TP-Int2 interventions in Study 2. While the liver and serum carotenoid profile largely reflected the dietary carotenoid composition, we did not detect phytoene in prostate or tumor tissue, as our laboratory has previously reported for TRAMP mice.[135]

Animal characteristics

TRAMP mice were enrolled onto each study at 4.0 ± 0.1 and 4.2 ±0.1 weeks of age in Study 1 and 2, respectively. Mice in Study 1 were a week older at castration than Study 2 (13.1 ± 0.1 weeks compared to 12.1 ± 0.1 weeks). There were no differences between the body weights at euthanasia across treatment groups in either study (Figure D.1). Additionally, there were no differences in the weight of the prostate tumor, liver,

epididymal adipose tissue, lungs, individual prostatic lobes and total prostate weight within studies (Table D.1).

Tumor incidence and metastases

Cancer incidence is displayed in Table 4.3. 76% of the animals in Study 1 and 77% of the animals in Study 2 developed histologically confirmed moderately or poorly differentiated adenocarcinoma with no differences between treatment conditions (p=0.82 in Study 1 and p=0.56 in Study 2). Expression of neuroendocrine features represented by synaptophysin immunohistochemistry, was expressed in 33% or 42% in the tumors from Studies 1 and 2 respectively, with no differences by treatment group. Metastatic spread was visually assessed at necropsy and lesions were confirmed by pathology. For both studies, the statistical analysis of distant metastatic disease was not possible for any site due to the low incidence of metastases observed (Table 4.4). The most common site of metastases was to the lymph node, which occurred in 4-30% of all animals. Approximately 78% of the metastases in Study 1 and 95% of the metastases in Study 2 stained positive for SV-40.

In vivo CRPC tumor growth

70% of mice in Study 1 and 66% of mice in Study 2 developed lesions that were detected by ultrasound and were eligible for *in vivo* growth analyses. Overall, ultrasound estimates for *in vivo* tumor volume were strongly correlated with tumor weight at necropsy (R^2 =0.76 and 0.94, p<0.001 for both Study 1 and 2, respectively) (Figures 4.3A and 4.3B). For both Study 1 and Study 2, no differences in tumor-free survival between the treatment groups (time to appearance of the first ultrasound-detected lesion) from the

time of castration (12-14 weeks of age) were noted in Study 1 (log rank p=0.91) and Study 2 (log rank p=0.70). These results remained the same even after using proportional hazards regression controlling for age and weight at castration.

The initial tumor volume (week 0) was not different between treatment groups for mice on either study (p=0.22 and p=0.28 for Study 1 and 2, respectively). The mean tumor volume at detection was approximately 50 mm^3 in Study 1 (Figure 4.3C) and 20 mm^3 in Study 2 (Figure 4.3D). The differences between tumor volume by treatment in the final week of the analysis (week 4) were also not different in either study (p=0.07 and p=0.87 for Study 1 and 2, respectively) compared to each respective control (Figure 4.3E and Figure 4.3F). Individual tumor growth curves can be found in Figure D.2.

Due to the large variability in the *in vivo* tumor volumes, tumor volumes were transformed by their natural logarithms. Regression analysis of *in vivo* log-transformed tumor volumes are shown in Table 4.5. In Study 1, a significant effect of time was noted (b=0.87, p<.0001), indicating that the log-transformed tumor volume increased with time (Table 4.5). The tests for interaction effects indicated that the slopes for TP-Int1 and TP-L were not different from that of the control group (b=-0.04, p=0.73 and b=-0.03, p=0.76, respectively). The main effect of TP-Int1 treatment on log-transformed tumor volume was significant, with a beta coefficient of -0.43 (p=0.04), relative to Con-L. This corresponds to a 35% decrease in the actual tumor volume (mm^3) in TP-Int1 compared to Con-L over the 5-week interval of tumor growth. The main effect of TP-L on log-transformed tumor volume and Con-L at Week 0 was not significant (b=-0.34, p=0.11). Tests of the interaction effects between treatments and time indicated that the slopes for TP-Int1 and

TP-L were not different from Con-L (b=-0.04, p=0.73 and b=-0.03, p=0.76, respectively), indicating no differences in tumor growth rates.

Similar to Study 1, a significant effect of time was noted (b=1.21, p<0.0001) in Study 2, indicating that the mean log-transformed tumor volume increased with time. Likewise, no interaction effects between treatment and time were found, indicating that the tumor growth rate for Con-Int did not differ from TP-Int (b=0.02, p=0.79) or Lyc-Int (b=0.01, p=0.83). Unlike Study 1, there were no main effects of dietary treatment, indicating that Con-Int did not differ from TP-Int2 (b=0.29, p=0.44) and Lyc-Int (b=0.35, p=0.38). Sensitivity analyses were conducted for both studies by using a weighted least squares approach and by removing extreme observations. However, the results of these analyses were unchanged.

Discussion

Men undergoing ADT as a component of curative multimodality therapy or for advanced or metastatic disease frequently consume supplements, many containing lycopene or tomato components, or increase their intake of tomato products, in hope of improving therapeutic outcome. There is currently a lack of quality pre-clinical research or clinical trials supporting the hypothesis that tomato products or lycopene enhance benefits of therapeutic interventions such as ADT. The present studies address this key gap in the scientific literature using a well-controlled and established TRAMP system with relevant physiological exposure to tomato components and lycopene to quantify their potential to reduce the evolution of CRPC. We hypothesized that dietary tomato or

lycopene would reduce CRPC incidence and progression in the TRAMP model based on epidemiological and preclinical data. In contrast to the reduction of *de novo* murine prostate carcinogenesis accompanying tomato and lycopene consumption,[114,116,297] the dietary treatments provided after castration did not affect incidence of histopathologic cancer, tumor weight at necropsy, final tumor volume by ultrasound, and duration of tumor-free survival (evaluated by ultrasound).

Importantly, this study, like other murine experiments using similar dosages of lycopene or tomato products, resulted in blood concentrations that are relevant to what is observed in humans.[97,116,135,297] Although the dose provided in the diet of mice for tomato powder interventions (~3 mg lycopene/kg body weight) may seem at first glance very excessive or pharmacologic (201 mg of lycopene per day for a 70 kg male), this concentration is necessary in a mouse to achieve blood concentrations similar to humans due to the poor absorption of carotenoids in rodents.[308] The ranges of blood lycopene concentrations found in this and similar studies correlates well with blood concentrations in American males over the ranges that are associated with a significant reduction in risk for lethal PCa in the Health Professionals Follow Up Study (HPFS) prospective cohort trial and other studies.[95,231,309] Importantly, this dose is easy to achieve through the diet. A human equivalent dose of 3 mg/kg in mice translates to 17 mg per day (0.24 mg/kg).[310] This could be achieved with a half serving of tomato sauce (1/4 cup, 60 grams) per day. Men accumulate carotenoids in the prostate and prostate lycopene concentrations increase similar to blood concentrations.[311] This has been demonstrated in studies with daily intake of standard tomato products such as juice, soup, or sauce over several weeks.[122,312,313] Recent studies also demonstrate that blood and prostate concentrations

of lycopene after consuming tomato juice are related to specific genetic polymorphisms that can alter carotenoid absorption and metabolism.[59,314] Together, these studies indicate that the mice in our studies achieve blood and tissue concentrations relevant to humans. As a result, these data are particularly relevant to human dietary interventions and adds confidence to our findings.

Experimental models that closely recapitulate the physiology, molecular biology, and natural selective pressures of CRPC development more reliably estimate the efficacy of preventative or therapeutic strategies. The TRAMP model exhibits castration sensitivity similar to humans,[88] is immunocompetent, exhibits a predictable histological progression from low-grade hyperplasia to poorly differentiated adenocarcinoma (ultimately with clear neuroendocrine features) and local as well as distant metastasis.[89,315,316] Furthermore, transcriptional signatures of human and TRAMP prostate cancer are similar.[97,317] The TRAMP model is also characterized by dysfunction of Rb and p53 due to the SV40 transgene, thereby disrupting cell cycle control and promoting genomic instability; aberrations in TP53 and RB are transcriptional signatures of human CRPC.[316] These features of the TRAMP model, both in *de novo* carcinogenesis and in response to castration, fortify our confidence that our new findings are relevant to human CRPC. Our data suggest caution in advising men undergoing ADT that increased tomato or lycopene consumption will likely reduce the severity of their disease.

Ultrasound evaluation of the emergence of individual castrate-resistant tumors over five weeks provided a unique and insightful dimension to our studies. The plots of individual ultrasound-derived tumor volumes (Figure D.2) displayed extreme heterogeneity in the growth rate of CRPC tumors. This variation was observed regardless

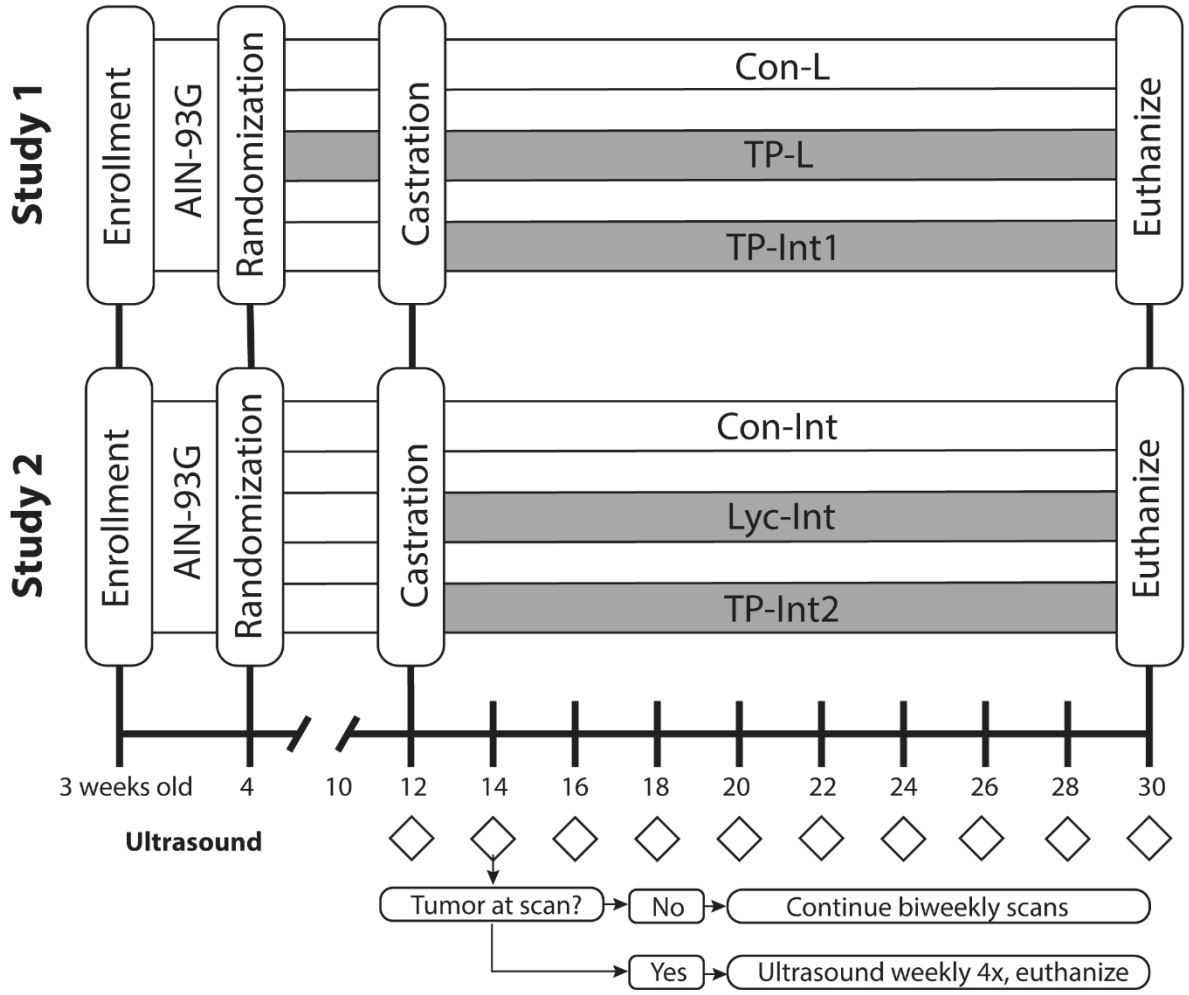
of dietary treatment and ranged between 10- and 100-fold. Remarkably, this heterogeneity is similar to the over 10-fold variation that is seen in the rate of progression for men failing initial ADT.[318] Although the sample sizes of the present experiments are large compared to other preclinical studies, this observed variation in tumor growth rates makes it difficult to detect modest changes in tumor growth rate due to dietary treatments. CRPC tumors that grow despite ADT typically maintain activity of androgen-mediated pathways, often through sustained androgen receptor signaling.[36] There are many pathways for PCa to progress to CRPC such as mutations affecting the function of the androgen receptor, affinity for alternative ligands, activation complementary growth promoting signaling pathways, and others.[36,319] It is likely that the specific mutational spectrum of individual CRPC lesions underlies the large variation in progression rates.

Of the very few studies of PCa progression[227,320] or CRPC[244,253,321], none have been sufficiently powered or adequately controlled. A recent systematic review of preclinical studies found that most eligible studies reported inhibitory effects of tomato or lycopene treatment on androgen-related outcomes.[322] Lycopene, in addition to other tomato bioactives, may affect tumor progression after castration by modifying inflammatory status; [103,104] androgen and growth factor signaling;[97] apoptosis;[104,111,112] and cell cycle progression.[104,111,112] Tomatoes contain other potentially beneficial carotenoids, and bioactive compounds that may reduce prostate tumorigenesis,[31] and some studies suggest that the whole fruit may be more effective than lycopene alone.[14] Further studies are needed that investigate the molecular profiles of CRPC tumors to determine if TP or lycopene feeding differentially affects specific molecular subtypes of CRPC.

In conclusion, our studies of tomato products in a well-characterized murine model of prostate carcinogenesis are relevant to a key issue for men with PCa undergoing ADT. The emergence and progression of CRPC was not altered by tomato or lycopene consumption. As science progresses, the role that dietary tomato or lycopene has on the growth and progression of specific molecular subtypes of CRPC may be explored with the rise of personalized nutrition and cancer treatment plans. Although data from these studies did not display a benefit from tomato consumption following castration (ADT), a recent single-blind, randomized, pilot trial of 32 men on ADT found that adherence to a diet and exercise-based lifestyle intervention shows promise for countering and/or reversing adverse effects of ADT.[323] Our findings in a model that is relevant to the evolution of human PCa with blood concentrations similar to human epidemiological literature suggest that men should focus on other fitness and healthy dietary guidelines (such as the Dietary Guidelines for America 2015) as they begin ADT rather than focus upon supplements of nutrients or other bioactives until a benefit has been demonstrated by well-designed experimental studies.

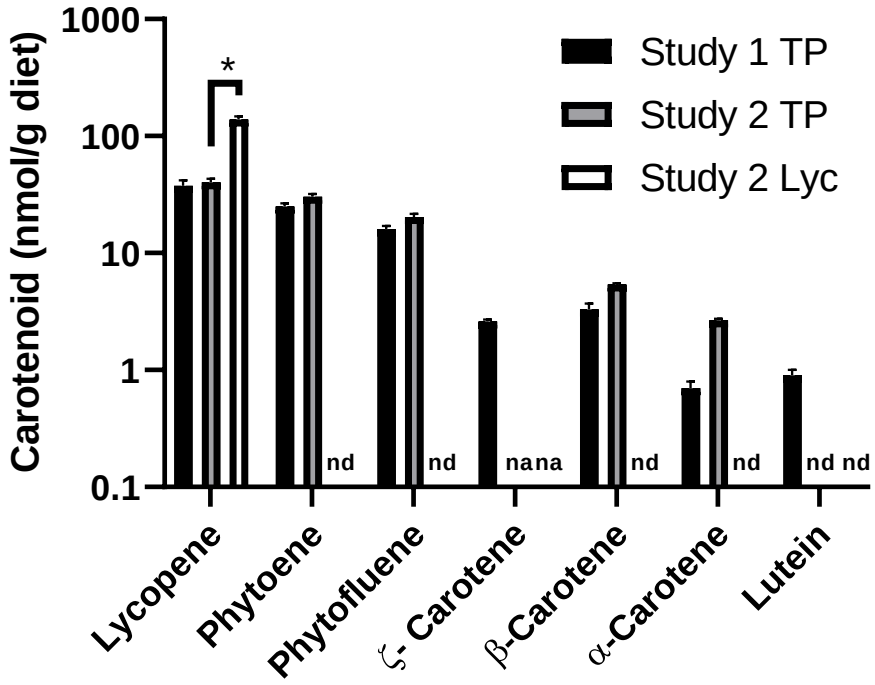
Figures and tables for Chapter 4

Figure 4.1: Study designs



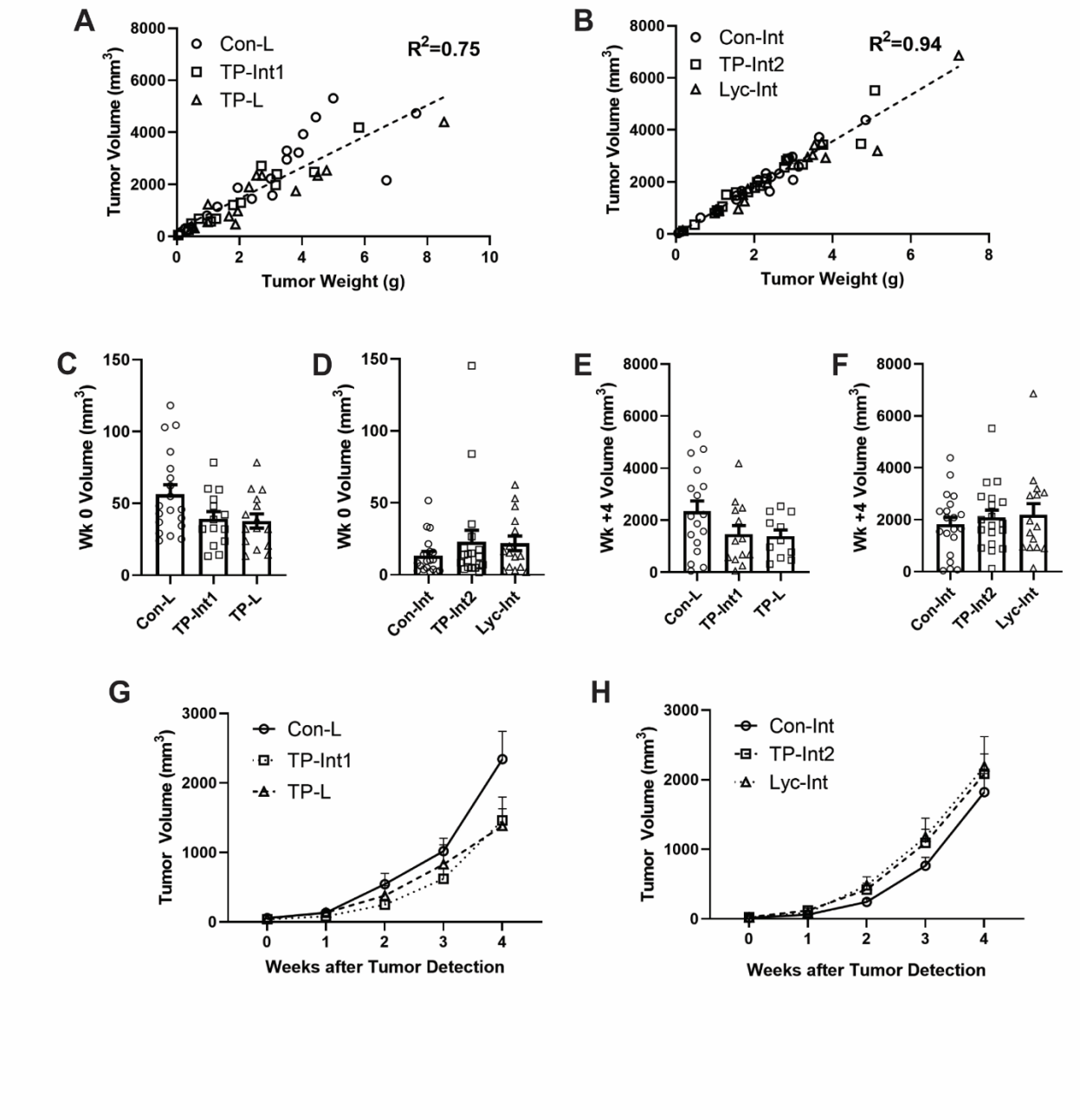
TRAMP mice were randomized onto a dietary treatment groups after weaning (4 weeks) or after castration. In Study 2, Con-Int and TP-Int2 mice received diets containing placebo beadlets. Prostates were monitored biweekly for tumor occurrence by ultrasound beginning at 10 weeks of age. After tumor detection, mice were scanned four additional times (+4 weekly ultrasound scans) to track changes in tumor volume. Mice without tumors detected by ultrasound were euthanized at 30 weeks of age.

Figure 4.2: Dietary carotenoid composition



Carotenoid composition of the tomato (TP) and lycopene (Lyc) diets. Data are mean concentration ± SEM across 6-7 diet batches with 2-3 replicates/batch. Data are presented as nmol/g diet. nd, not detected, carotenoid concentration was below the limit of detection (0.005 nmol carotenoid/ g diet); na, not analyzed. Lycopene content was compared between Study 2 TP and Study 2 Lyc by t-. test. * indicates statistical significance ($p<0.001$). Control diets were not included due to their lack of carotenoids in the diet.

Figure 4.3: Tumor volume correlations and means



Correlation of tumor weight at necropsy vs. tumor volume at final ultrasound scan *in vivo*. A, Study 1; B, Study 2. C-F, *in vivo* tumor volume. C. Study 1, week 0 (tumor detection); D, Study 2, week 0 (tumor detection); E, Study 1, week +4; F, Study 2, week +4. G, Study 1 *in vivo* tumor volumes. Week of tumor detection is set at 0. H, Study 2 *in vivo* tumor volumes. Week of tumor detection is set at 0. Data are expressed as means ± SEM. For clarity, only the upper error bars are displayed. Individual points represent individual tumors.

Table 4.1. Composition of experimental diets.

	g/kg diet		
	Control	10% Tomato	Lycopene
Cornstarch	390	363	390
Maltodextrin	130	105	130
Sucrose	98	97	98
Casein	196	177	196
Cellulose	49	41	49
AIN-93G mineral mix	34	34	34
AIN-93G vitamin mix	10	10	10
L-Cystine	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5
Soybean oil	70	68	70
Lyophilized tomato paste	0	100	0
10% Lycopene beadlets	0	0	0.47
Placebo beadlets[1]	0.47	0.47	0
Water	18	0	18
kcal/g diet [2]	3.9	3.8	3.9

[1]Placebo beadlets were included only in the Control and Tomato diets of Study 2.

[2]Calculated.

Table 4.2. Carotenoid accumulation in castrated TRAMP mice fed experimental diets.

		n	Lycopene[1]	Phytoene	Phytofluene	ζ-Carotene	α-Carotene
Serum	TP-L	6	569.1 ± 97.6[2]	69.1 ± 10.7	141.5 ± 44.4	n.a.[3]	n.a.
nmol/L	TP-Int1	6	551.1 ± 96.8	59.1 ± 12.3	135.7 ± 8.6	n.a.	n.a.
	TP-Int2	23	285.4 ± 21.8	109.9 ± 7.4	242.4 ± 26.5	n.a.	n.d.
	Lyc-Int	21	345.8 ± 35.0	n.d.[4]	n.d.	n.a.	n.d.
Liver	TP-L	12	15.7 ± 2.1	5.6 ± 0.9	10.5 ± 1.5	1.7 ± 0.2	0.03 ± <0.01
nmol/g	TP-Int1	11	17.4 ± 2.1	5.7 ± 0.7	11.2 ± 1.5	1.6 ± 0.2	0.03 ± <0.01
	TP-Int2	29	10.1 ± 0.5	4.7 ± 0.5	13.8 ± 1.1	n.a.	n.d.
	Lyc-Int	25	8.5 ± 1.1	n.d.	n.d.	n.a.	n.d.
Prostate	TP-L	2[5]	0.38 ± 0.09	n.d.	0.15 ± 0.02	0.34 ± 0.07	n.d.
nmol/g	TP-Int1	2	0.37 ± 0.04	n.d.	0.12 ± 0.01	0.32 ± 0.03	n.d.
	TP-Int2	1[5]	0.68	n.d.	0.51	n.a.	n.d.
	Lyc-Int	1	1.50	n.d.	n.d.	n.a.	n.d.
Tumor	TP-L	3	0.09 ± 0.01	n.d.	0.04 ± 0.01	0.04 ± <0.01	n.d.
nmol/g	TP-Int1	4	0.12 ± 0.02	n.d.	0.13 ± 0.04	0.06 ± 0.01	n.d.
	TP-Int2	6	0.05 ± 0.02	n.d.	0.14 ± 0.02	n.a.	n.d.
	Lyc-Int	6	0.06 ± 0.02	n.d.	n.d.	n.a.	n.d.

Table 4.2. (Continued)

[1]Total lycopene (sum of all *trans* and *cis* stereoisomers).

[2]All values represent the mean ± SEM. By t-test, there were no significant differences between TP treatments (Study 1, TP-L and TP-Int1) in tissue accumulation of any carotenoid. In Study 2 (TP-Int2, Lyc-Int), no significant differences were observed between lycopene concentrations TP-Int2 and Lyp-Int.

[3]n.a., not analyzed.

[4]n.d., not detected. Concentration was below the limit of detection. The limit of detection was 0.015 nmol of each carotenoid per gram tissue. Lutein and β-Carotene were analyzed, but not detected in any tissue.

[5]Prostate concentrations from Study 1 (TP-L and TP-Int1) are means of two pools of 8 to 12 mice each, while prostates for Study 2 (TP-Int2 and Lyc-Int) are the average of 2-5 animals that were pooled.

Table 4.3. Incidence of histologically confirmed prostate adenocarcinoma or neuroendocrine carcinoma

	Adenocarcinoma (WD-PD)[1]		Prostatic Lesion Score (% total)[2]					Neuroendocrine carcinoma[1]	
Treatment	+ / total n	**%**	NSL	PIN	WD	MD	PD	+ / total n	**%**
Study 1									
Con-L	19/25	76	16	8	8	4	64	4/16	25
TP-Int1	18/23	78	17	4	4	13	61	5/13	38
TP-L	17/22	77	18	5	5	27	45	4/10	40
Study 2									
Con-Int	21/27	78	22	0	0	4	74	9/21	29
TP-Int2	22/28	79	18	4	0	0	79	14/22	55
Lyc-Int	20/27	74	26	0	0	11	63	8/19	44

[1]Fisher's exact test between control and respective treatment were not significant for adenocarcinoma and neuroendocrine incidence in Study 1 (Con-L, TP-Int1, TP-L) or Study 2 (Con-Int, TP-Int2, Lyc-Int).

[2]Cancer incidence was evaluated by stage by a trained veterinary pathologist. NSL, no significant lesion; PIN, prostatic intraepithelial neoplasia; WD, well differentiated adenocarcinoma; MD, moderately differentiated adenocarcinoma; PD, poorly differentiated carcinoma.

[3]n, total mice available for comparison within each treatment group. Data are provided as the number (+) and percent (%) of mice positive for a designated pathology within each treatment group.

Table 4.4. Incidence of distant metastases

Treatment		Lymph Nodes		Liver		Lungs		Kidney	
	n[1]	**+**[2]	**%**	**+**	**%**	**+**	**%**	**+**	**%**
Study 1									
Con-L	25	5	20	2	8	7	28	5	20
TP-Int1	23	1	4	0	0	1	5	1	5
TP-L	23	7	30	0	0	1	4	1	4
Study 2									
Con-Int	29	5	17	1	3	2	7	1	3
TP-Int2	29	8	28	1	3	2	7	2	7
Lyc-Int	28	7	25	1	4	3	11	4	14

[1]n, total mice available for comparison within each treatment group.

[2]Data are provided as the number (+) and percent (%) of mice positive for a designated pathology within each treatment group.

Table 4.5. Generalized linear mixed model regression analyses of *in vivo* tumor growth.

	Estimate[1]	SE	t	p
Study 1				
Intercept	3.89	0.14	27.45	**<0.0001**
Time	0.87	0.07	12.54	**<0.0001**
Treatment				
TP-L	-0.34	0.21	-1.65	0.105
TP-Int1	-0.43	0.20	-2.13	**0.038**
Time*Treatment				
TP-L*time	-0.03	0.10	-0.31	0.760
TP-Int1*time	-0.04	0.10	-0.35	0.726
Study 2				
Intercept	2.44	0.27	9.11	**<0.0001**
Time	1.21	0.05	22.27	**<0.0001**
Treatment				
TP-Int2	0.29	0.37	0.78	0.440
Lyc-Int	0.35	0.40	0.89	0.380
Time*Treatment				
TP-Int2*time	0.02	0.08	0.26	0.792
Lyc-Int*time	0.02	0.08	0.21	0.832

[1]Estimates and standard errors (SE) are expressed as: Intercept, the natural log of tumor volume (in mm^3) at detection (week 0); Time, the natural log of the relative increase in tumor volume in one week in the control group; Treatment, the natural log of the relative difference between the *in vivo* tumor volume at week 0 of a respective treatment group and the control group; Time*Treatment, the natural log of the relative difference between the tumor growth rate of a respective treatment group and the tumor growth rate of the control group).

Chapter 5: Radiation efficacy was not altered by a 10% tomato powder diet in TRAMP mice

[e]*Abstract*

Background: Tomatoes contain carotenoids and other potent antioxidants that may protect the surrounding tissue from the detrimental effects of external beam radiation therapy (EBRT), while reducing rates of prostate carcinogenesis. We hypothesized that a diet containing lyophilized tomato paste (TP) would not reduce apoptosis or cell death within the prostate tumor following EBRT, while reducing apoptosis and cell death within surrounding tissues.

Methods: To evaluate this hypothesis, we conducted a Pilot and Diet study. In the Pilot study, male TRansgenic Adenocarcinoma of the Mouse Prostate (TRAMP) mice (n=18) were provided a powdered AIN-93G diet at 4 weeks of age. In the Diet study, TRAMP (n=76) were provided a control diet or a modified AIN-93G diet containing 10% TP (w/w) at 4 weeks of age. In both studies, prostates were monitored by ultrasound. Once tumors reached a volume of 500 mm^3 (Pilot study), 1000 mm^3 (Diet study) or at 24 weeks of age, the caudal half of the mouse was irradiated with 7.5 Gy (Rad) or 0 Gy (sham) with a Cobalt-60 source. In the Pilot study, mice were euthanized after 0, 24, or 72 hours following radiation or sham treatment. Based on the Pilot study, mice were euthanized 24

[e] Please note that additional experiments and analyses are delayed due to COVID-19 related closures or are currently underway that might alter the final results and conclusions of this chapter.

hours after radiation or sham treatment in the Diet study. Antioxidants (carotenoids and α-tocopherol) were measured by high performance liquid chromatography (HPLC). Tissues were assessed by a pathologist for radiation-induced changes (hematoxylin and eosin) and apoptosis (cleaved caspase-3). Inflammatory markers (C-reactive protein, IL-6, IL-17A, TNFα, IFNγ, and IL-10) in serum and selected tissues were evaluated by ELISA or a Bio-Plex multiplex immunoassay.

Results: The caudal half of the animal received a dose of 7.3 Gy of radiation, while the cranial half of the animal received 0.6 Gy. Cell death and apoptosis scores within the tumor increased with radiation treatments compared to sham-treated mice, while no changes occurred in the surrounding prostate. Following radiation treatment, circulating levels of lycopene (52% lower), phytoene (26% lower), and α-tocopherol (22% lower) were lower compared to sham treated mice ($p<0.05$). Likewise, serum levels of TNFα (50% lower), INFγ (35% lower), IL-6 (35% lower), IL-17a (35% lower), and IL-10 (22% lower) were reduced with radiation compared to sham-treated mice ($p<0.05$). However, tissue levels of carotenoids and α-tocopherol were not modified by radiation.

Conclusions: Collectively, these data indicate that radiation therapy with TP was not inferior to radiation alone; however, TP did not improve any of the measured endpoints.

Introduction

Prostate cancer (PCa) is the second leading cause of cancer related deaths among men in western countries.[4] If PCa is detected early, common therapeutic options include: active surveillance, surgery (prostatectomy), external beam radiation therapy

(EBRT) or chemotherapy.[11-13] In particular, EBRT has become more precise and novel methods of delivering the radiation dose have been proposed to improve patient outcomes. Despite improved treatment, approximately 60-69% of men who receive EBRT develop biochemical recurrence.[14] In clinical practice, the maximal dose is typically limited by potential for damage to the surrounding (non-tumor) tissues. As the dose of radiation increases, oxidative damage within the tumor and surrounding tissue increases and the number of required fractions (doses) decreases.[18-21] Acute and chronic toxic effects such as gastroenteritis, bowel dysfunction, sexual dysfunction, decrease the clinical effectiveness of EBRT. For example, radiation therapy is typically accompanied with bowel and sexual dysfunction,[15] and importantly (but less common), secondary tumors and PCa progression.[16,17]

Following PCa diagnosis, patients often seek information about food and supplements that may improve their response to therapies, quality of life, and survival. Tomatoes and lycopene are two of the most frequently mentioned foods or supplements by social media, lay press, and purveyors of alternative therapy as having a protective effect on prostate cancer activity. Lycopene, in addition to other tomato bioactives, may affect tumor progression after castration by modifying inflammatory status; [103,104] androgen and growth factor signaling;[97] apoptosis;[104,111,112] and cell cycle progression.[104,111,112] Lycopene and other carotenoids that are present within tomatoes are potent antioxidants that may improve natural defenses and scavenge free radicals generated during radiolysis. Anti-oxidants may quench singlet molecular oxygen (reactive oxygen species, ROS). Reducing ROS may suppress the highly pro-inflammatory environment following EBRT and further affect apoptosis within the tumor. Decreasing

ROS in normal tissues is radioprotective and may lead to fewer adverse effects and protection from oxidative damage that is caused by radiation therapy.[324,325] Although these data suggest that PCa may provide a benefit to men who are undergoing treatment for radiation therapy, the lack of pre-clinical data prevent more definitive trials from occurring and remains a gap in the literature.

The goal of this study was to address the hypothesis that tomato feeding would protect surrounding tissues from inflammatory damage, without affecting radiation-induced damage in the tumor in the TRansgenic Adenocarcinoma of the Mouse Prostate model. To evaluate this hypothesis, we conducted 2 studies. The first study was a pilot study, which established the timing for termination of mice following radiation and to establish the radiation dose that would be used in a dietary study. The second study aimed to evaluate the potential of lifelong consumption of tomato powder to alter radiation-induced damage (apoptosis and double-stranded DNA-breaks) in the prostate tumor and surrounding tissues. To our knowledge, this is the first study to evaluate the efficacy of dietary tomato or lycopene on inflammatory-induced changes to the tumor and surrounding organs within an animal model.

Materials and Methods

Diets

Many of the methods used for this section are similar to the Materials and Methods for Chapter 4 (pages: 87-94). Briefly, tomato paste (Contadina®, San Francisco, CA) was purchased from a local supermarket in September 2014, July 2015, and in April 2016 and by lyophilized in a VirTis Freezemobile 12SL/Unitop 600 SL freeze dryer (SP Scientific,

Warminster, PA). Dried yield was ~25% of wet mass. Lyophilized tomato paste (TP) was ground to fine powder in a tabletop food processor, transferred to resealable gallon bags (air removed), and kept in the dark at -20˚C until diet mixing.

Two experimental diets were used in this study: a powdered, AIN-93G-based control diet and the same diet modified to contain 10% (w/w) lyophilized tomato paste (TP). Ingredients were mixed using a commercial mixer (Hobart, Illinois. USA). Proximate analysis was performed on the 100% tomato paste powder and diet formulas were balanced for total energy, carbohydrates, protein, fat, fiber, and moisture. New diets were formulated every 2 months. Each batch of diet was analyzed for carotenoid content by high-performance liquid chromatography (HPLC).

Mouse breeding, genotyping, and housing

The details for breeding, genotyping, and housing are the same as Chapter 4 (pages 88-89). The University of Illinois Laboratory Institutional Animal Care and Use Committee reviewed and approved all experimental procedures (Protocol 18029). Male C57BL/6-Tg(TRAMP)8247Ng/J (C57BL/6 TRAMP+/-), female C57BL/6J, and female FVB/NJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). A breeding colony was maintained with crosses of C57BL/6J females and C57BL/6 TRAMP+/- males. Male F1 offspring of FVB/NJ females and C57BL/6 TRAMP+/- males were used for the study. Tail DNA of pups was isolated with Extract-N-Amp™ Tissue PCR kits (Sigma-Aldrich, St. Louis, MO) and mice were genotyped to confirm transgene presence. Males carrying the probasin:SV40-Tag transgene (hereafter referred to as TRAMP mice) were weaned at 3 weeks of age and enrolled into the study via rolling admission. Mice

were housed under controlled conditions (12-hour light/dark cycle, 22˚C, 55% humidity), weighed weekly, and diet was added 3 times per week.

Pilot study

TRAMP mice will be randomized onto an AIN-93G control diet (n=18) at four weeks of age. Power analyses (power=0.80 and α=0.05) have indicated that 15 animals per group will be sufficient to detect a 40% change in oxidative damage in surrounding tissues.[326] Beginning at 10 weeks of age, weekly *in vivo* ultrasound imaging was used for longitudinal screening and tumor volume measurement. Inhalation isoflurane was used for general anesthesia. Ultrasonic scans were obtained through the ventral body wall while in dorsal recumbency on a heated table using the Vevo 2100 pre-clinical ultrasonic imaging platform (VisualSonics, Inc., Toronto, Canada). Scans were conducted in 3D B-mode, and frames were collected in a caudal to cranial direction at intervals of approximately 0.152 mm. Serial 2D image slices were used to generate prostatic or tumor volume estimates as previously described.[303]

Once tumors reached a volume of 500 mm^3 or at 24 weeks of age without a detected tumor by ultrasound, mice were irradiated with 7.5 Gy by a Cobolt-60 source at a dose rate of 0.22 Gy/min (Theratron-780 Isocentric teletherapy) (n=12) or 0 Gy (sham treatment – Sham, n=6). The radiation was collimated to protect the cranial half of the mouse. Inhalation isoflurane was used for general anesthesia. 7.5 Gy of radiation was selected to be equivalent to one quarter of a human patient's hypofractionated therapeutic dose. The dose was calculated to be equivalent to 3.5 Gy that was delivered in 5 fractions (equivalent to 25% of a human's hypofractionated total dose).[327-330] This dose was confirmed by dosimeters that were placed under the caudal and cranial halves of the

animal. The prostate received 7.3 Gy of radiation, while the cranial half of the animal received a dose of 0.6 Gy (Figure E.1). Non-radiated sham animals underwent the same procedure as the radiation treated animals without radiation exposure. Animals were euthanized 0, 24, or 72 hours after radiation or sham treatments. The study designs for the pilot and diet studies can be found in Figure 5.1.

Diet study

TRAMP mice were acclimated to the AIN-93G control diet from weaning at 3 to 4 weeks of age and randomized to consume control diet (Con, n=35) or 10% TP (n=39). Similar to the pilot study, prostates were scanned by ultrasound beginning at 10 weeks of age, biweekly (every 2 weeks) for longitudinal screening and tumor volume measurement. Inhalation isoflurane was used for general anesthesia. Mice with prostate tumors identified were switched from biweekly ultrasound screening to weekly ultrasound scans in order to measure tumor volume. Once tumors reached a volume of 1000 mm^3 or at 24 weeks of age without a detected tumor by ultrasound, mice were irradiated with 7.5 Gy by a Cobolt-60 source at a dose rate of 0.22 Gy/min (Rad, n=19; TP-Rad, n=18) or 0 Gy (sham treatment – Sham, n=16; TP-Sham, n=20). Animals were euthanized 24 hours after radiation or sham treatments.

Necropsy

TRAMP mice were exsanguinated by cardiac puncture under deep anesthesia followed by cervical dislocation. When possible, the prostate was dissected into individual lobes (anterior, dorsal, lateral and ventral). Suspected malignant prostate masses (tumors) were dissected from the remaining prostate. Individual prostate lobes, malignant

prostate tumors, seminal vesicles, liver, lungs, and epididymal adipose tissue were weighed and snap frozen in liquid N_2 and stored at -80˚C for future analysis. Gross metastases to the lungs, liver, kidneys, urethra, and regional lymph nodes (medial iliac and lumbar aortic, when present) were identified by visual inspection, and tissues were fixed in 10% neutral-buffered formalin for 12 to 24 hours and held in 70% aqueous ethanol until paraffin embedding.

Histopathology and immunohistochemistry

Tissues were embedded in paraffin and 4-μm-thick sections were stained with hematoxylin and eosin (H&E). A blinded examiner evaluated the bladder, urethra, each lobe of the prostate, the prostate lesion (tumor), small intestines, kidney, and liver for morphological changes including cell death, edema, and infiltration of macrophages. Additionally, this blinded examiner (MAW) also evaluated the extent and severity of neoplasia in prostate and tumor sections as previously described.[304] Metastases were confirmed by H&E and SV-40 staining. Apoptosis was quantified by cleaved caspase-3 staining (Cell Signaling Technology, MA). Double-stranded DNA damage was quantified by γ-H2A.X (ABCAM, MA).

Carotenoid measurement

Diet and tissue carotenoids were extracted and analyzed by HPLC as previously described.[62,305] Approximately 25 mg diet, 300 mg tumor tissue, 200 μL serum, and 100 mg liver tissue were used for analysis. Anterior prostates from 10 mice per treatment condition (one lobe per mouse) were pooled into two individual replicates.

Cytokine analysis

All samples were stored at −80°C until the day of the assay .The serum levels of IL-1β, IFNγ, TNFα, IL-6, IL-10, and IL-17A cytokine profiles were measured using a Bio-Plex multiplex assay (Bio-Rad, Hercules, CA) as previously described.[331] Serum levels of C Reactive Protein (CRP) was assessed by an ELISA following the manufacturer's instruction (ABCAM, MA). Portions of the liver, epididymal adipose tissue, anterior prostate, and prostate tumor were homogenized in PBS and frozen at -20°C overnight. Samples were thawed and centrifuged at 2000 x g for 15 minutes. The supernatant was transferred to a new tube, and TNFα was quantified by an ELISA following the manufacturer's instructions (Thermo-Fisher, MA).

Statistical Analysis

Statistical analyses were performed in SAS version 9.4 (SAS Institute, Inc., Cary, NC) and GraphPad Prism 8.4 for Windows. Tissue weights, serum and tissue cytokines, and gene expression were compared using one or two-way ANOVA with multiple-comparison adjustments by Tukey's method. Data were log transformed when assumptions of normality were not met. Histological scores were assessed by Wilcoxon's Rank-Sum Test whenever two samples were present at a given time point. Otherwise, the nonparametric Kruskal-Wallis test was performed with multiple-comparisons by Dunn's test. Unless otherwise stated, a p-value < 0.05 was considered statistically significant. All values are reported as mean ± standard error of mean (SEM).

Results

Animal characteristics of the Pilot study

There were no differences between the body weights at euthanasia across time points that were evaluated in (Figure E.1). Additionally, there were no differences in the epididymal adipose tissue, lungs, heart, and total prostate weight within studies (Table E.1). Following radiation treatment (24- or 72-hours following radiation), the mean weight of the spleen decreased by 50% ($p<0.001$). Mean testicular weight also decreased by 10% after 72 hours ($p<0.01$). Interestingly, mean PCa tumor weight appeared to be 68% lower than sham-treated mice 72 hours following irradiation compared to sham-treated mice; however, the number of tumors for this comparison were low (Table E.2).

By 24 weeks of age, 100% of mice developed histologically-confirmed prostate adenocarcinoma Table 5.1. Histological evaluation of tumors suggests that radiation increased the median necrosis and apoptosis scores within the tumor from 0-1% in sham treated mice to 25-50% (24 and 72 hours post radiation) (Table 5.2). Statistical evaluation of these scores were not appropriate because of the low number of mice with tumors in the 72-hour group (n=2). No significant lesions or morphological changes were noted in the small intestine, bladder, urethra, prostate, or liver with radiation exposure (data not shown). 72 hours after radiation, serum C-reactive protein levels were 50% lower than the sham-treated and 24-hour post radiation groups (Figure 5.2A). Based on this information, we selected 24 hours for the Diet study.

Animal characteristics of the Diet study

Unlike the Pilot Study where 100% of mice developed histologically-confirmed prostate adenocarcinoma, 59% of all mice (59% of control-fed and 59% of TP-fed mice)

developed PCa by 24 weeks of age (Table 5.1). There were no differences between the body weights at euthanasia across in any dietary or treatment condition (Figure E.1). There also were no differences in the liver, epididymal adipose tissue, lungs, heart, testes, prostate tumor weight, individual prostatic lobes and total prostate weight within studies (Table E.2). For the spleen, there was a main effect of radiation such that irradiated mice had lower mean spleen weights compared to sham-treated mice ($p<0.0001$).

Tumor apoptosis and cell death

Apoptosis and necrosis were evaluated by a trained veterinary pathologist (M.A.W.) within the PCa tumor and surrounding prostate tissue. There were no significant lesions or morphological changes within the urethra, bladder, small intestine, or liver as a result of diet or radiation treatments (data not shown). Cell death scores within the tumor are described in Table 5.2, and cleaved caspase-3 scores for apoptosis are described in Table 5.3. Within the tumor, the median score for cell death (necrosis and apoptosis) was between 1.5 and 2.5 for all treatment conditions representing that 10-25% of the tumor was necrotic or apoptotic. Neither diet nor treatment altered cell death within the tumor ($p=0.33$).

Within the tumor, the median cleaved caspase-3 score (apoptosis) increased from 3 in sham-treated mice to 4 in irradiated mice. A score of 3 indicates that <50% of lobules have positively stained cells, while a score of 4 indicates that > 50% of cells lobules have positive cells. Apoptosis was not modified by treatment or diet within the anterior ($p=0.85$) or ventral prostates ($p=0.35$). Interestingly, the median score for cleaved caspase-3 (apoptosis) was altered in the dorsolateral prostate by diet. The median score for control-fed mice within the dorsolateral prostate was 2, while the median score for tomato-fed

mice was 1 (Table 5.3) (p=0.0017). This might be an important finding due to the fact that most PCa tumors arise within the dorsolateral lobes of the prostate.[89] Future studies are needed to confirm if apoptosis in specifics lobes of the prostate are sensitive to the effects of tomato carotenoids.

Carotenoid and α-tocopherol content of diet and accumulation in tissues

The ingredients for each diet are described in Table E.1. The carotenoid composition of the tomato diet is shown in Figure 5.3, while carotenoid accumulation is shown in Table 5.3. Lycopene was the predominant carotenoid (39 nmol/g, 21 mg/kg, 49% of total carotenoids) in the TP diet. The AIN-93G base for both diets also provided ~120 nmol/g (75 IU) of α-tocopherol. The profile of carotenoids in the liver and serum largely reflected the composition of the tomato diet for sham treated mice. Radiation decreased serum concentrations of lycopene ($p<0.0001$), phytoene ($p<0.01$), and α-tocopherol ($p<0.01$) compared to sham treated mice by 48%, 26%, and 22% respectively (Table 5.3). Although lycopene and other antioxidants were lowered following radiation in the serum, tissue concentrations in the liver and tumor were not modified by radiation. Antioxidants in the prostate was not statistically compared; however, mean α-tocopherol concentrations within the prostate were numerically 42% lower in irradiated mice compared to sham-treated mice.

Inflammatory markers

Four pro-inflammatory (TNFα, IFNγ, IL-17a, and IL-6) cytokines and one anti-inflammatory (IL-10) cytokine were measured in the serum. There were no main effects of diet on the measured cytokines. Interestingly, radiation decreased circulating TNFα

(p<0.0001), IFNγ (p<0.001), IL-6 (p<0.01), IL-17a (p<0.01), and IL-10 (p=0.01) concentrations by 50%, 35%, 35%, 35% and 22%, respectively (Figure 5.4). TNFα concentrations within the liver (p=0.30), adipose tissue (p=0.22), prostate (p=0.27), and tumor (p=0.63) were not significantly modified by diet or radiation (Figure 5.4). C-reactive protein concentrations within the serum were also not modified by diet (p=0.24) (Figure 5.2B).

Discussion

Males that undergo treatment for PCa often consume supplements containing lycopene or tomato components with the goal of improving their therapeutic outcomes. However, no epidemiological, pre-clinical, or clinical trials have evaluated the potential for tomato consumption to modify the tumor and surrounding tissues' response to therapy. The current study addresses a gap in the literature by evaluating the hypothesis that tomato feeding can protect the tissues surrounding the prostate tumor from radiation-induced damage. The current studies are the first to evaluate the interactions between tomatoes and radiation within a pre-clinical model. We hypothesized that a diet containing lyophilized tomato paste (TP) would not reduce apoptosis or cell death within the prostate tumor following EBRT, while reducing apoptosis and cell death within surrounding tissues. The data from this study indicates that radiation therapy in TRAMP mice consuming TP was as effective at destroying tumor tissue as radiation therapy in mice receiving no TP. Contrary to our hypothesis, however, TP did not affect any of the measured endpoints in surrounding tissues.

Life-long tomato consumption is hypothesized to prime a tumor to be more radiosensitive by initially stimulating changes that make tumors less aggressive and by altering pathways involved in cancer progression (halting cell cycle status,[104,111,112] increasing apoptosis,[104,111,112] reducing growth factor signaling,[97] and reducing angiogenesis).[95,107,108] There is currently only one small study in humans (n=17) to our knowledge that evaluated the interactions between tomato consumption and EBRT.[332] The aforementioned study supplemented men with 0 oz (control) of tomato juice, 4 oz, 8 oz, or 12 oz of tomato juice during radiation therapy.[332] The goal of that study was to evaluate the tolerance and adverse events associated with tomato supplementation. No adverse events were noted with lycopene consumption indicating its safety. Although the sample size was small, an interesting finding from this study was that higher serum lycopene concentrations were associated with less cachexia and improved therapeutic outcomes among patients treated with EBRT.[332] In the United States, more than 85% of dietary lycopene comes from tomato products, which suggests that lycopene could be a surrogate biomarker of tomato consumption.[44,45,133] Larger clinical and pre-clinical studies are necessary to determine the role that tomato consumption might have on EBRT.

Tomato consumption increases circulating carotenoid concentrations, which may result in increased antioxidant protection and decreased inflammation.[102,333] Radiation either damages DNA directly or indirectly by forming reactive oxygen species (ROS) that react with organelles to disrupt normal cellular metabolism. About two-thirds of damage by irradiation is caused indirectly through action of ROS.[334] Lycopene and other tomato carotenoids are potent antioxidants that may act as a buffer against ROS caused by EBRT in normal (non-tumor tissue). It is possible that lycopene and other tomato

carotenoids may decrease the efficacy of EBRT by quenching ROS in the tumor before substantial damage occurs to the DNA.[334,335] In the current study, circulating levels of antioxidants (such as lycopene and α-tocopherol) were lowered by 35-50% radiation after 24 hours. Without an adequate supply of antioxidants from the diet, such as lycopene and α-tocopherol, serum and tissue concentrations would rapidly become depleted. It is likely that surrounding tissues would be more heavily damaged through indirect activity of ROS. As injuries in these tissues accumulate, clinical acute and late stage toxicities may occur leading to digestive, urinary, bowel, or sexual dysfunction.[15]

Cell death and apoptosis scores after radiation were not altered by the consumption of TP. This suggests that radiation therapy with TP consumption was not inferior to radiation alone. The use of antioxidants during radiation therapy is controversial because it is hypothesized that antioxidants can also protect the targeted tissue from the effects of radiation.[336-339] This is important because many men who are diagnosed with PCa choose to improve their diet after diagnosis. Although consumption of fruits and vegetables generally increase following PCa diagnosis, tomato consumption remains relatively constant (<15% increase after diagnosis) following diagnosis.[187] Studies such as this are important for men who are being treated with treatments such as EBRT. The amount of lycopene that was consumed in this study is easy to achieve through the diet. A human equivalent dose of 3 mg/kg in mice translates to 17 mg per day (0.24 mg/kg).[310] This could be achieved with a half serving of tomato sauce (1/4 cup, 60 grams) per day. Men accumulate carotenoids in the prostate and prostate lycopene concentrations increase similar to blood concentrations.[311] The range of blood lycopene concentrations found in this study is also correlated with a significant reduction in risk for lethal PCa in

the Health Professionals Follow Up Study (HPFS) prospective cohort trial and other studies.[95,231,309]

Mice were irradiated with 7.5 Gy of half-body radiation. This dose is potentially lethal to cells in the bone marrow and spleen due to substantial increases in apoptosis and cell death.[340-343] Likewise, mice that were irradiated in the current studies displayed a 36% lower spleen weight compared to sham-treated mice after 24 hours. This decrease might be indicative of the hematopoietic and lympohcytic cells that have undergone apoptosis within the spleen. Reductions in spleen volume/weight are also noted in people who have their spleen irradiated for cancer or other reasons.[344] The spleen and bone marrow are both components of the lymphatic system.[345,346] Circulating concentrations of cytokines within the serum were lower in mice that were treated with radiation compared to sham-treated mice in this study. This has been observed in other studies.[343,347] We hypothesized that the observed decrease in cytokines are due to the effect of radiation on the lymphatic system (e.g. spleen, bone marrow, and lymph nodes) that were exposed to radiation.

These studies exhibit strengths that contribute to the literature. Among the possible animal models that were available, we selected the TRAMP model. This model is well established and exhibits a predictable histological progression from low-grade hyperplasia to poorly-differentiated adenocarcinoma (many with clear neuroendocrine features) and local as well as distant metastasis, resembling development of the human PCa.[89] Carotenoids and other dietary compounds also are distributed to organs in a similar manner to humans. In this study, we used lyophilized tomato paste (TP), which provides a vehicle for more than a single active compound (supplement). This provided

potential for greater synergistic effects of each nutrient present within the whole food. Tomato paste is readily available, convenient, and safe for a consumer to purchase and consume during radiation therapy. To monitor tumor emergence in this study, ultrasound provided us with a more precise *in vivo* estimation of tumor emergence and growth. In Chapter 4, tumor weight and volume were tightly correlated, and within the current study, we were able to detect tumors as small as 10 mm^3.

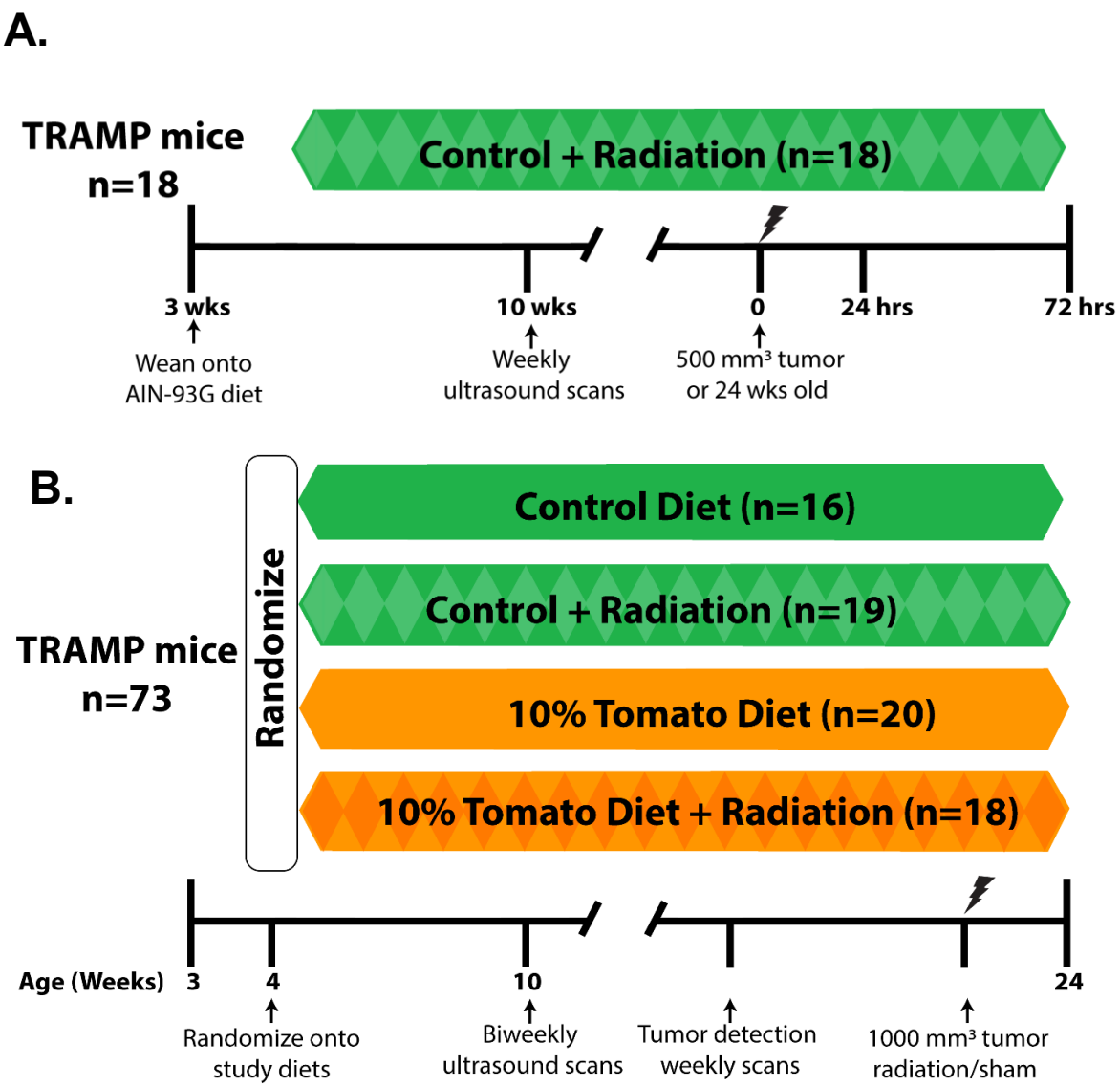
Despite these strengths, this study also has several limitations that should be considered. This study used a single dose of 7.5 Gy of radiation. While this dose was calculated to be equivalent to 25% of a patient's total dose of hypofractionated EBRT (3.5 Gy for 5 days),[327,329,330,348] many acute toxicities occur following weeks after the onset of the treatment.[349,350] It is possible that the accumulation of damage from several doses might have a different effect on damage to the tumor and surrounding tissue compared to a single dose. Future studies will be needed to determine if TP alters tumor cell death over a prolonged period of time. Additionally, this study only tested a single level of dietary TP throughout the lifespan. The results of this study are more generalizable to a man that consumes TP through their lifespan than one that changes their dietary pattern following PCa diagnosis. Although multiple doses were not evaluated, men accumulate lycopene in the prostate with moderate doses and short periods of tomato consumption.[312] A randomized clinical trial (RCT) of 66 men found that one serving of tomato dishes containing 200 g of Hunt's Spaghetti Sauce (providing 30 mg of lycopene per day) was sufficient to increase plasma and prostatic lycopene concentrations in 3 weeks.[122]

To our knowledge, this is the first pre-clinical study to evaluate the potential for tomato consumption to alter the response to EBRT within the tumor and within the

surrounding tissues. This study provides preliminary data that suggest that tomato consumption with radiation therapy does not affect the damage occurring within the tumor or surrounding tissue. This study suggests that tomato consumption does not inhibit the main treatment effects of EBRT in prostate tumors; however, this study does not support the hypothesis that TP protects the surrounding tissues from the harmful effects of EBRT. As a result, improving tomato consumption can safely serve as a low-cost method of decreasing the risk of developing PCa without affecting the efficacy of subsequent treatment regiments.[127,132] Future clinical studies should evaluate the concentration of lycopene in the blood as a potential biomarker of tomato consumption to determine whether increased circulating lycopene correlates positively or negatively with treatment-related outcomes.

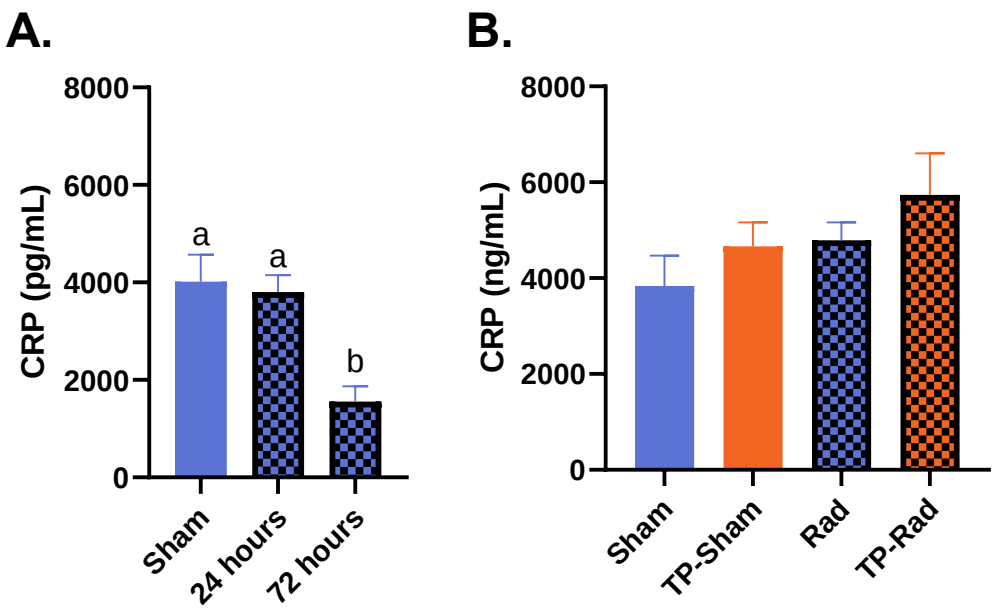
Figures and tables in Chapter 5

Figure 5.1 Study Design



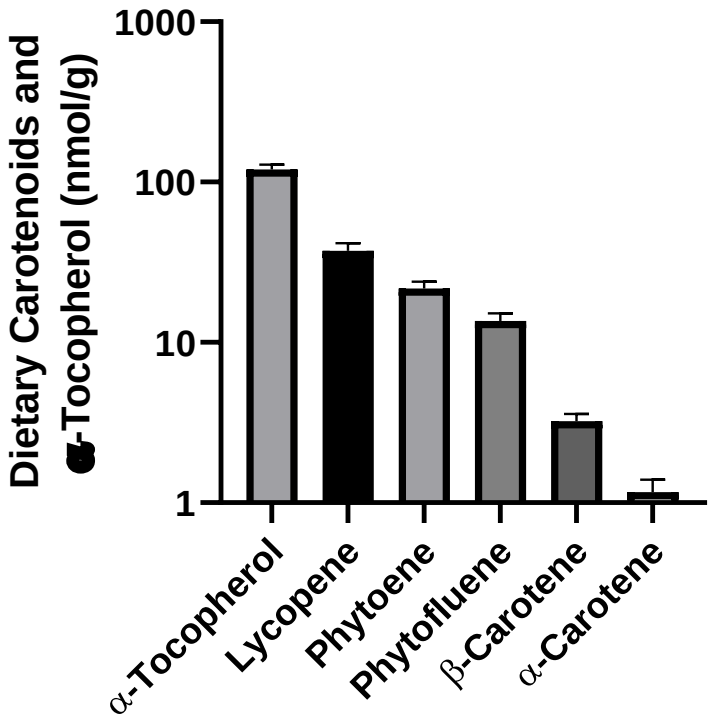
TRAMP mice were randomized onto a dietary treatment groups after weaning (4 weeks). Prostates were monitored weekly (Pilot Study – Figure A) or biweekly (Diet study – Figure B) for tumor occurrence by ultrasound beginning at 10 weeks of age. After tumor detection, mice were scanned weekly by ultrasound until tumors reached a volume of 500 (Pilot Study) mm^3 or 1000 mm^3 (Diet study). At this volume, the caudal half of the animal was irradiated with 7.5 Gy by a Co-60 source. Mice were euthanized 24- or 72-hours following radiation or after a sham treatment.

Figure 5.2 Serum C-reactive protein levels in TRAMP mice



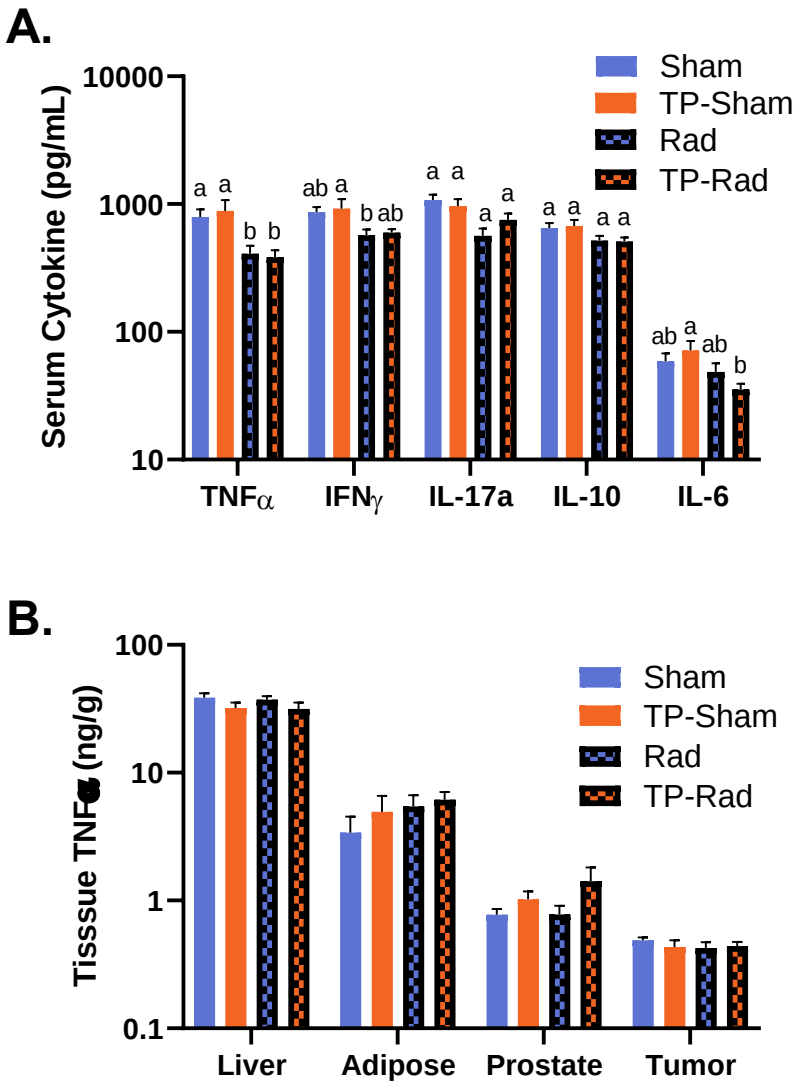
Data are represented as mean ± SEM. Means with different letters signify that means differ (one-way ANOVA followed by Tukey's post hoc test, $p<0.05$). A) Serum concentrations of C-reactive protein (CRP) in the Pilot Study (n=6 per group, performed in duplicate). B) Serum concentrations of CRP in the Diet study (n=8-13 per group, performed in duplicate).

Figure 5.3 Carotenoid and α-tocopherol content in the tomato powder (TP) diet



Data are mean concentration ± SEM across 3 diet batches with 2 replicates/batch. Data are presented as nmol/g diet. Control diets were not included due to their lack of carotenoids in the diet.

Figure 5.4 Concentration of cytokines in tissues 24 hours after irradiation



Data are represented as mean ± SEM. Means with different letters signify that means differ (one-way ANOVA followed by Tukey's post hoc test, $p<0.05$). A) Serum concentrations of cytokines (n=8-13 per group). B) Tissues levels of TNFα (n=6 per group).

Table 5.1 Incidence of adenocarcinoma in TRAMP mice

	Adenocarcinoma (WD-PD)		Prostatic Lesion Score (% total)				
Treatment	+ / total n	**%**	NSL	PIN	WD	MD	PD
Pilot Study	14/14	100					
Sham	4/4	100	0	0	50	0	50
24 Hours	6/6	100	0	0	33	17	50
72 Hours	4/4	100	0	0	50	50	0
Diet Study	41/70	59					
Sham	11/14	79	0	21	21	7	50
TP-Sham	12/20	60	0	40	15	0	45
Rad	8/19	42	0	58	11	11	21
TP-Rad	10/17	59	0	41	24	0	35

Data are provided as the number (+) and percent (%) of mice positive for a designated pathology within each treatment group. Cancer incidence was evaluated by stage by a trained veterinary pathologist. NSL, no significant lesion; PIN, prostatic intraepithelial neoplasia; WD, well differentiated adenocarcinoma; MD, moderately differentiated adenocarcinoma; PD, poorly differentiated carcinoma. Histologically confirmed adenocarcinoma occurred in 59% of control and tomato fed animals (equally distributed by diet).

Table 5.2 Tumor apoptosis and necrosis within TRAMP tumors

	eval/total n[1]	Mean ± SEM	Median	preliminary p-value*
Pilot Study				
Sham	3/3	1.7 ± 1.2	1	0.5667
24 hours	4/4	3.0 ± 0.9	3	
72 hours	2/2	3.0 ± 1	3	
Diet Study				
Sham	7/9	1.6 ± 0.3	2	0.3264
TP-Sham	8/9	1.6 ± 0.3	1.5	
Rad	4/6	2.3 ± 0.9	2.5	
TP-Rad	7/7	2.4 ± 0.4	2	

Data are expressed as means ± standard error and as a median. There were no significant differences in medians (Kruskal-Wallis test). A score of 0 represents 0-1% cell death (apoptosis and necrosis); a score of 1 represents up to 10% cell death; a score of 2 represents 10-25% cell death; a score of 3 represents 25-50% cell death; and a score of 4 represents greater than 50% of the cells are dead.

[1]the eval/total n represents the number of slides that have been completed of the total number of slides that exist for this endpoint. Due to the closures from COVID-19, data from these slides are not currently included.

Table 5.3 Cleaved caspase-3 evaluation for apoptosis

	Eval/total n[1]	Mean ± SEM	Median	preliminary p-value*
Tumor				
Sham	1/9	3	3	0.2557
TP-Sham	5/9	2.8 ± 0.5	3	
Rad	5/6	4.2 ± 0.5	4	
TP-Rad	7/7	4.0 ± 0.4	4	
Dorsolateral Prostate				
Sham	5/12	1.6 ± 0.2	2[a]	**0.0017**
TP-Sham	10/17	0.8 ± 0.1	1[b]	
Rad	8/19	1.6 ± 0.2	2[a]	
TP-Rad	9/17	1.0 ± 0.0	1[ab†]	
Ventral Prostate				
Sham	4/7	0.5 ± 0.3	0.5	0.8283
TP-Sham	7/12	0.3 ± 0.2	0	
Rad	6/13	0.3 ± 0.3	0	
TP-Rad	4/12	0.3 ± 0.3	0	
Anterior Prostate				
Sham	14/16	1.4 ± 0.4	1	0.3514
TP-Sham	17/20	1.2 ± 0.2	1	
Rad	14/19	1.2 ± 0.3	1	
TP-Rad	13/18	0.8 ± 0.2	1	

Data are expressed as means ± standard error and as a median. Medians with different letters are significantly different from each other (Kruskal-Wallis test followed by Dunn's multiple comparisons test; p-value <0.05). † signifies that TP-Rad compared to Rad in the dorsolateral prostate was trending toward significance (p=0.068). A Cleaved caspase-3 score of 0 represents no increase over background (0-1 positive cell/lobular profile); a score of 1 represents a focal increase above background (<25% lobular profiles with 2-5); a score of 2 represents "widespread" increases above background (>25% lobular profiles with 2-5); a score of 3 represents a "massive" increase above background (>25% lobular profiles with >5 positive cells per profile); a score of 4 represents that 50% lobules affected, with > 50% having 6-10 or more positive cells, generally associated with foci of cell death; occasional aggregates of positive cells; and a score of 5 represents 50% lobules affected, with >50% having 10+ cells per lobule centered around the edges of foci of cell death plus scattered aggregates of positive cells.

[1]the eval/total n represents the number of slides that have been completed of the total number of slides that exist for this endpoint. Due to lab closures from COVID-19, data from these slides are not currently included.

Table 5.4 Carotenoid and α-tocopherol accumulation in TRAMP mice fed tomato diets.

		n	Lycopene[1]	Phytoene	Phytofluene	α-Carotene	α-Tocopherol
Serum	TP-Sham	17	865 ± 84***	174 ± 12*	834 ± 94	n.d.	67,817 ± 4,711*
nmol/L	TP-Rad	13	456 ± 59	129 ± 11	618 ± 69	n.d.	53,120 ± 3,437
Liver	TP-Sham	11	16.9 ± 2.4	4.64 ± 0.59	27.4 ± 3.2	0.17 ± 0.06	181 ± 21
nmol/g	TP-Rad	12	23.1 ± 2.1	5.10 ± 0.74	31.4 ± 4.4	0.16 ± 0.07	188 ± 23
Prostate[2]	TP-Sham	2	0.44 ± 0.07	n.d.	0.40 ± 0.02	n.d.	8.52 ± 0.92
nmol/g	TP-Rad	2	0.72 ± 0.07	n.d.	0.46 ± 0.14	n.d.	4.96 ± 2.14
Tumor	TP-Sham	4	0.28 ± 0.02	2.74 ± 0.77	0.49 ± 0.08**	n.d.	18.0 ± 1.9
nmol/g	TP-Rad	4	0.46 ± 0.12	3.26 ± 1.32	0.90 ± 0.07	n.d.	23.0 ± 3.6

[1]Total lycopene (sum of all *trans* and *cis* stereoisomers).
[2]Prostate concentrations are means of two pools with 5 anterior prostates (1 lobe per mouse) in each.
All values represent the mean ± SEM. * means differ, $p<0.05$ (t-test). ** means differ, $p<0.01$ (t-test). *** means differ, $p<0.001$ (t-test). n.d., not detected. Concentration was below the limit of detection. The limit of detection was 0.015 nmol of each carotenoid per gram tissue. Lutein and β-Carotene were analyzed, but not detected in any tissue.

Chapter 6: Summary and future directions

Prostate cancer is the most frequently diagnosed cancer among men in the United States. Although preventing prostate cancer (PCa) is preferable to treating PCa, patients seeking information about foods and supplements might already have cancer. Importantly, the prostate microenvironment is not the same prior to cancer initiation, during PCa promotion/ progression, and during treatment. There is a growing body of pre-clinical and epidemiological evidence that suggests that tomato products and compounds (such as lycopene) decrease PCa incidence; however, no studies (to our knowledge) have evaluated the interactions between the consumption of tomato products and common therapeutic approaches for PCa. Elucidating whether dietary approaches can improve therapeutic outcomes should positively affect treatment selection and recommendations for patients. In this dissertation, we sought to clarify the epidemiological associations between tomatoes (and lycopene) and PCa incidence, and to evaluate the role of tomato feeding in an animal model that underwent two common treatment approaches. More specifically, the aims of this dissertation were to:

1. Evaluate the epidemiological associations between the exposures of tomatoes or lycopene and risk of prostate cancer in the epidemiological literature.
2. Determine the potential of tomato or lycopene interventions to reduce the growth and progression of CRPC in the TRAMP model
3. Evaluate the potential for lyophilized tomato powder to reduce radiation-induced apoptosis and death in prostate tumors and surrounding tissues the TRAMP model

Despite potential benefits that have been reported in the literature, epidemiological studies evaluating carotenoids and their role in protection from prostate carcinogenesis and progression have yielded mixed results. In Chapters 2 and 3, we aimed to evaluate the associations between tomatoes or lycopene and PCa incidence. The development of PCa is complex and is influenced by a combination of genetic, hormonal and environmental factors.[97] Diet is one of the most modifiable risk factors for PCa. Many epidemiological studies have investigated tomato products and their association with PCa carcinogenesis. Despite much positive data, studies have yielded mixed results. Lycopene, a lipophilic carotenoid that gives some fruits and vegetables their red color, is a primary bioactive component in tomatoes.[44,45] We hypothesized that increased consumption of tomato products or lycopene would result in a lower risk of PCa in the epidemiological literature. To elucidate these associations, two dose-response meta-analyses were conducted.

The first meta-analysis (Chapter 2) focused on the interactions between tomato products and risk of PCa. Thirty studies related to tomato consumption and PCa risk were included in the meta-analysis, which summarized data from 24,222 cases and 260,461 participants. Higher total tomato consumption was associated with a reduced risk of PCa (RR=0.81, p=0.001). This association was supported by several dose-response meta-analyses of tomato products. In particular, we found that bioavailability of lycopene was important. Raw tomato consumption was not associated with a decreased risk of PCa (p=0.49), while cooked tomatoes and sauces (sources with high lycopene bioavailability) were associated with a decreased risk of PCa (p=0.03).

The second meta-analysis (Chapter 3) evaluated associations between lycopene and PCa incidence. Forty-two studies were included in the analysis, which included 43,851 cases of PCa reported from 692,012 participants. Similar to the whole food product, increased lycopene consumption (RR=0.88, p=0.02) and blood concentrations (RR=0.88, p=0.02) were associated with a decreased risk of PCa. These associations were also supported by dose-response associations. There was an estimated 9% reduced risk of PCa by consuming 100 grams/week of cooked tomatoes in Chapter 2. For an equivalent dose of lycopene (22 mg lycopene in 100 grams of tomato puree according to the USDA Nutrient Database), tomatoes were more effective than lycopene at reducing PCa risk (9% compared to 1.6%). The greater benefit from tomato products may be due to interactions between potentially beneficial bioactive compounds in tomatoes. These meta-analyses support the hypothesis that tomato products or lycopene reduce prostate carcinogenesis.

Although data from these studies support significant and consistent reductions in the risk of developing prostate cancer with higher consumption of tomato products, very few studies reported risk associations by stage or grade of PCa. Many studies included in our meta-analyses did not include the grade or stage of PCa and contained only a small number of cases with advanced stages of PCa. Due to the small number of studies that investigated advanced PCa, it is difficult to obtain adequate estimates of the associations between tomatoes products and risk of advanced stages of PCa. A pooled analysis of prospective cohort studies found that pizza with tomato sauce was significantly associated with a reduced risk of lethal PCa.[126] Another pooled analysis of prospective cohort studies found that increased circulating lycopene was significantly associated with

a reduced risk of advanced PCa.[94] Combined, these pooled analyses suggest that tomato consumption may be associated with advanced PCa. A better understanding of the associations between the stage or grade of PCa and carotenoid status may lead to a reduced progression of PCa. More studies are needed in order to elucidate the associations and potential benefits of tomato consumption on advanced PCa risk.

As the prostate tumor is treated with common approaches, such external beam radiation therapy (EBRT) or androgen deprivation therapy (ADT), many changes occur within the prostate microenvironment. If PCa is detected early, common therapeutic options include: active surveillance, surgery (prostatectomy), radiation therapy and/or chemotherapy.[11,12] Despite primary treatment, approximately 30% of men suffer tumor recurrence.[31] These men, along with men diagnosed with advanced or metastatic cancer, usually undergo ADT.[31,32] This aggressive and often lethal stage of PCa occurs after the prostate tumor acquires mutations to sustain growth despite ADT.

In Chapter 4, we aimed to determine whether dietary lyophilized tomato powder (TP) or lycopene would be capable of affecting the growth of castration-resistant prostate cancer (CRPC). We hypothesized that tomato or lycopene products would reduce the growth and emergence of CRPC. We administered a physiologically relevant dose of a commercially available tomato food (Contadina® brand tomato paste), in order to maximize translatability of this animal study. A human equivalent dose of 3 mg/kg in mice translates to 17 mg per day (0.24 mg/kg).[310] This could be achieved with a half serving of tomato sauce (1/4 cup, 60 grams) per day. Men accumulate carotenoids in the prostate and prostate lycopene concentrations increase similar to blood concentrations.[311] Contrary to our hypothesis, dietary treatments after castration did not significantly reduce

cancer incidence, time to tumor detection, or final tumor weight. This is the first study to evaluate the potential for a dietary intervention to modify the growth of CRPC tumors.

Ultrasound evaluation of the emergence of individual castrate resistant tumors over five weeks provided a unique and insightful dimension to our studies. The individual growth rates of CRPC tumors were extremely heterogeneous regardless of dietary treatment and ranged between 10- and 100-fold. This heterogeneity is similar to the over 10-fold variation that is seen in the rate of progression for men failing initial ADT.[318] Although the sample sizes of the present experiments are large compared to other preclinical studies, this observed variation in tumor growth rates would make it difficult to detect modest effects of the dietary treatments on tumor growth rates.

CRPC tumors that grow despite ADT typically maintain activity of androgen-mediated pathways, often through sustained androgen receptor signaling.[36] There are many pathways for PCa to progress to CRPC such as mutations affecting the function of the androgen receptor, affinity for alternative ligands, activation complementary growth promoting signaling pathways, and others.[36,319] It is likely that the specific mutational spectrum of individual CRPC lesions underlies the large variation in progression rates. Depending on the acquired mutation, some tumors might have been more responsive to a dietary tomato or lycopene intervention. A recent systematic review of preclinical studies found that most eligible studies reported inhibitory effects of tomato or lycopene treatment on androgen-related outcomes.[322] Lycopene, and other tomato bioactives may improve the effectiveness of ADT by reducing tumor promoting inflammation,[104] androgen/growth factor signaling,[97,110] survival,[104,111,112] and cell cycle progression.[104,111,112] [14] Further

studies are needed that investigate the molecular profiles of CRPC tumors to determine if TP or lycopene feeding differentially affects specific molecular subtypes of CRPC.

Approximately 80% of new PCa cases are eligible for EBRT. In Chapter 5, we hypothesized that dietary lyophilized tomato powder (TP) would not reduce the efficacy of external beam radiation therapy (EBRT) in the prostate tumor, but would protect surrounding tissues from radiation-induced damage. Lycopene and other tomato carotenoids are potent antioxidants that may act as a buffer against elevated ROS caused by EBRT in normal (non-tumor tissue); however, it is also possible that lycopene and other tomato carotenoids may reduce the efficacy of EBRT by quenching ROS in the tumor before substantial damage occurs to the DNA.[334,335]

Following radiation treatment, circulating levels of lycopene, phytoene, and α-tocopherol decreased compared to sham treated mice. Supporting our hypothesis, cell death and apoptosis scores within the tumor increased with radiation treatments compared to sham-treated mice. Contrary to our hypothesis, no changes in apoptosis scores occurred in the surrounding prostate. Collectively, these data indicate that radiation therapy in TRAMP mice consuming TP was as effective at destroying tumor tissue as radiation therapy in mice receiving no TP. However, TP did not affect any of the measured endpoints. This is important because many men do not alter their consumption of tomato products following diagnosis (unlike other fruits and vegetables). Additional studies will be needed to determine whether tomato consumption can improve radiation efficacy in the tumor or surrounding tissues when radiation is provided at different doses in multiple fractions.

In conclusion, this work has revealed consistent inverse associations with both tomato products and lycopene with the risk of prostate cancer incidence within published epidemiological studies. Our preclinical studies focusing on the potential of tomato products to improve PCa treatments (ADT and EBRT) found that the responses to treatment were not modified by a diet containing 10% TP or lycopene. While further work in animal models and humans is needed, it is notable that these are the first studies to evaluate the interactions between tomato products on common PCa treatments. Further studies should expand on these results and evaluate differences within the tumor microenvironment to determine if tomato products are more effective for specific subtypes or specific clonal populations of PCa. Data from these epidemiological and preclinical studies illuminate the importance of healthy dietary patterns throughout the lifespan given that it is much easier to modify the risk of developing PCa, rather than successfully treating existing PCa. These data are of great interest to patients who are diagnosed with PCa that seek out information about the types of foods or supplements that can improve their clinical outcomes. In conclusion, this dissertation has contributed novel findings to our understanding of the interactions between tomato products and PCa incidence, risk, and treatment.

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Appendix A: Supplemental tables for Chapter 1

Table A.1: Sources of dietary lycopene

Table A.2: Sources of dietary β-carotene

Table A.3: Sources of dietary α-carotene

Table A.4: Sources of dietary β-cryptoxanthin

Table A.5: Sources of dietary lutein and zeaxanthin

Table A.6: Average dietary intake and serum concentration of carotenoids

Table A.1 Sources of dietary lycopene

Food	Portion description	Portion weight (g)	Lycopene content per portion (mg)
Tomato juice, 100%	1 cup	248	22.4
Spaghetti sauce	½ cup	130	16.5
Watermelon, raw	1 medium wedge	286	13.0
Canned Stewed tomatoes	1 cup	255	10.4
Tomatoes, raw	1 medium	123	3.2
Grapefruit, raw	1 medium	256	2.9
Tomato catsup	1 tablespoon	15	1.8
Dried Papaya	1 strip	23	0.7

Adapted from the 2015-2016 USDA FNDDS

Table A.2 Sources of dietary β-carotene

Food	Portion description	Portion weight (g)	β-carotene content per portion (mg)
Carrot juice	1 cup	240	22.3
Baked sweet potato	1 medium	150	17.2
Cooked spinach	1 cup	190	13.7
Kale, cooked from frozen	1 cup frozen	130	11.4
Mustard greens, cooked from fresh	1 cup	140	10.3
Carrots, raw	1 cup, NFS	110	9.1
Parsley, raw	1 cup	60	3.0
Kale, raw	1 cup loosely packed	25	1.5

NFS refers to not further stated. Data were adapted from the 2015-2016 USDA FNDDS

Table A.3 Sources of dietary α-carotene

Food	Portion description	Portion weight (g)	α-carotene content per portion (mg)
Carrot juice	1 cup	240	10.4
Cooked pumpkin	1 cup, NFS	245	6.6
Carrots, raw	1 cup, NFS	110	3.8
Cooked winter squash	1 cup, NFS	245	1.7
Plantain, raw	1 medium	179	0.8
Pumpkin bread	1 slice	60	0.7
Mandarin oranges, canned	1 cup	189	0.4
Dandelion greens	1 cup	55	0.2

NFS represents not further stated. Data were adapted from the 2015-2016 USDA FNDDS

Table A.4 Sources of dietary β-cryptoxanthin

Food	Portion description	Portion weight (g)	β-cryptoxanthin content per portion (µg)
Persimmon, raw	1 persimmon	168	2,431
Papaya, raw	1 medium	304	1,791
Mandarin oranges, canned	1 cup	189	1,465
Red peppers, raw	1 medium	119	583
Tangerine, raw	1 medium	88	358
Papaya, dried	1 strip	23	224
Calamondin, raw	1 fruit	19	77
Kumquat, raw	1 kumquat	19	37

Adapted from the 2015-2016 USDA FNDDS

Table A.5 Sources of dietary lutein and zeaxanthin

Food	Portion description	Portion weight (g)	Lutein and Zeaxanthin content per portion (mg)
Spinach, cooked from frozen	1 cup	190	29.7
Kale, cooked from frozen	1 cup	130	25.4
Dandelion greens, raw	1 cup	55	7.5
Chard, raw	1 cup	36	4.0
Spinach, raw	1 cup	25	3.0
Kale, raw	1 cup	25	2.0
Broccoli, raw	1 cup	88	1.2
Egg yolk, cooked	1 large	17	0.2

Adapted from the 2015-2016 USDA FNDDS

Table A.6 Average dietary intake and serum concentration of carotenoids

Carotenoid	Diet[a] (μg/day)	Serum[b] (μg/dL)	Serum[b] (μmol/L)
Lycopene	4465 ± 101	43.01 ± 0.25[c]	0.804 ± 0.005[c]
α-carotene	366 ± 13	4.05 ± 0.07	0.075 ± 0.001
β-carotene	1910 ± 45	16.53 ± 0.24[d] 1.03 ± 0.14[e]	0.308 ± 0.004[d] 0.019 ± 0.0003[e]
Lutein + Zeaxanthin	1409 ± 43	15.78 ± 0.11	0.277 ± 0.002
β-cryptoxanthin	85.6 ± 2.4	11.19 ± 0.11	0.202 ± 0.002

[a]Data from 2015-2016 NHANES. Values represent means ± standard error.
[b]Data from 2005-2006 NHANES. Values represent means ± standard error.
[c]Value represents total lycopene (cis- and trans-isomers)
[d]Value represents trans-isomers for β-carotene
[e]Value represents cis-isomers for β-carotene

Appendix B: Supplemental table and figures for Chapter 2

Table B.1. List of items included in tomato foods category

Figure B.1. Forest plot for cumulative meta-analysis of highest vs lowest quantile of overall tomato consumption on PCa risk. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure B.2. Funnel plot for publication bias among studies investigating tomatoes and PCa risk. (A) total tomato consumption, (B) tomato foods; (C) cooked tomatoes and sauces, (D) raw tomatoes, (E) pizza and PCa (F) tomato consumption and risk of advanced PCa.

Figure B.3. Forest plot for cumulative meta-analysis of highest vs lowest quantile of consumption of tomato foods on PCa risk. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure B.4. Forest plot for cumulative meta-analysis of highest vs lowest quantile of consumption of cooked tomatoes and sauces on PCa risk. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure B.5. Forest plot for cumulative meta-analysis of highest vs lowest quantile of consumption of raw tomatoes on PCa risk. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure B.6. Forest plot for cumulative meta-analysis of highest vs lowest quantile of consumption of pizza on PCa risk. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

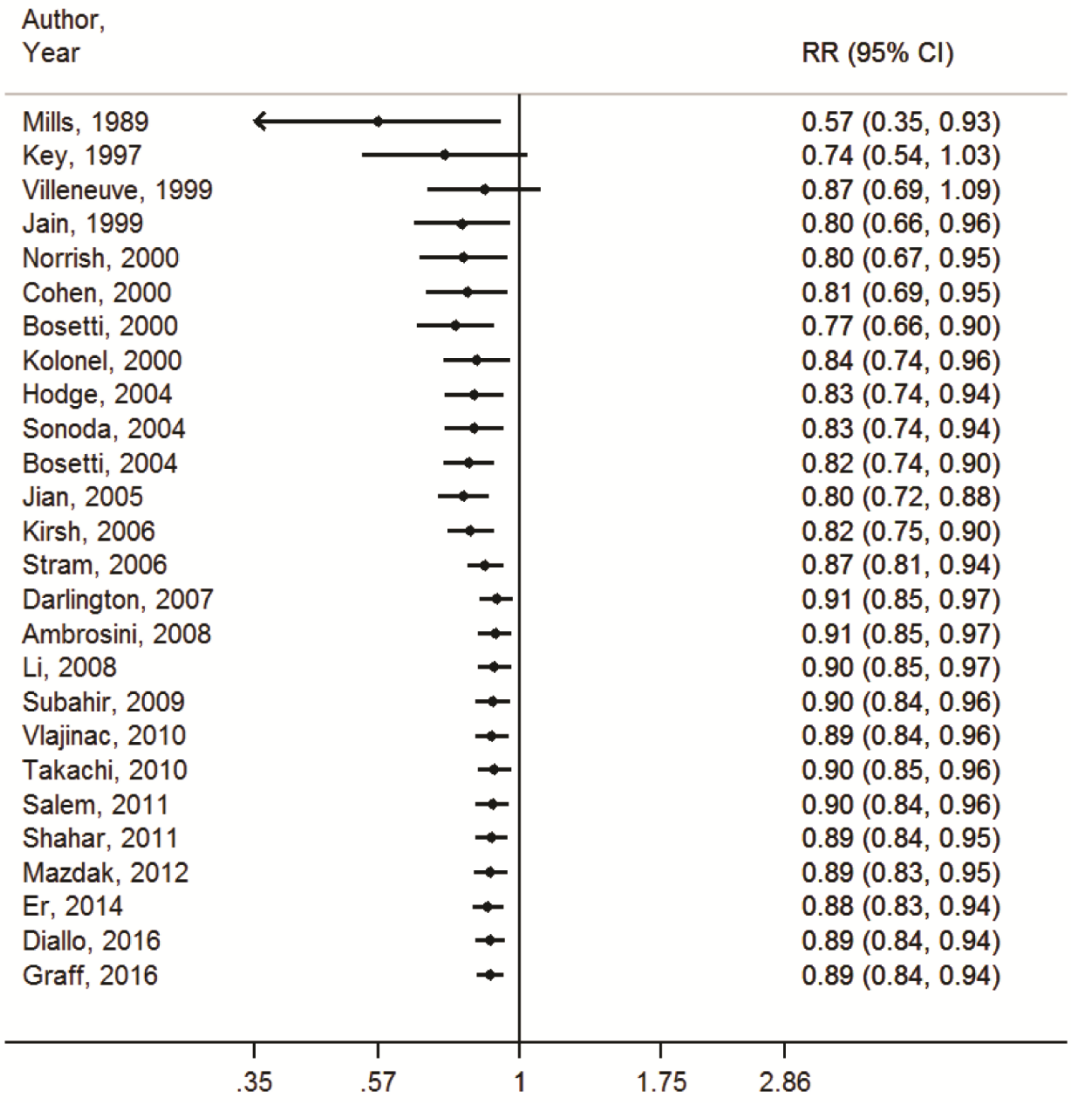
Figure B.7. Dose-response analysis of tomato consumption and PCa risk by study type. The solid black line is the nonlinear model curve for published relative risk and the two dotted lines are the corresponding 95% confidence intervals. The short-dashed line is the linear model curve for the published relative risks (RR); (A) cohort studies for total tomato consumption and risk of PCa; (B) case-control studies for total tomato consumption and risk of PCa; (C) cohort studies for tomato foods and risk of PCa; (D) case-control studies for tomato foods and risk of PCa; (E) cohort studies for cooked tomatoes & sauces and risk of PCa; (F) case-control studies for cooked tomatoes & sauces and risk of PCa; (G) cohort studies for raw tomatoes and risk of PCa; (H) case-control studies for raw tomatoes and risk of PCa.

Figure B.8. Dose-response analysis of tomato consumption and PCa risk before exclusion of one study. The solid black line is the nonlinear model curve for published relative risk and the two dotted lines are the corresponding 95% confidence intervals. The short-dashed line is the linear model curve for the published relative risks (RR); (A) total tomato consumption and risk of PCa; (B) case-control studies for total tomato consumption and risk of PCa; (C) tomato foods and risk of PCa; (D) case-control studies for tomato foods and risk of PCa.

Table B.1: Foods included in the tomato foods category

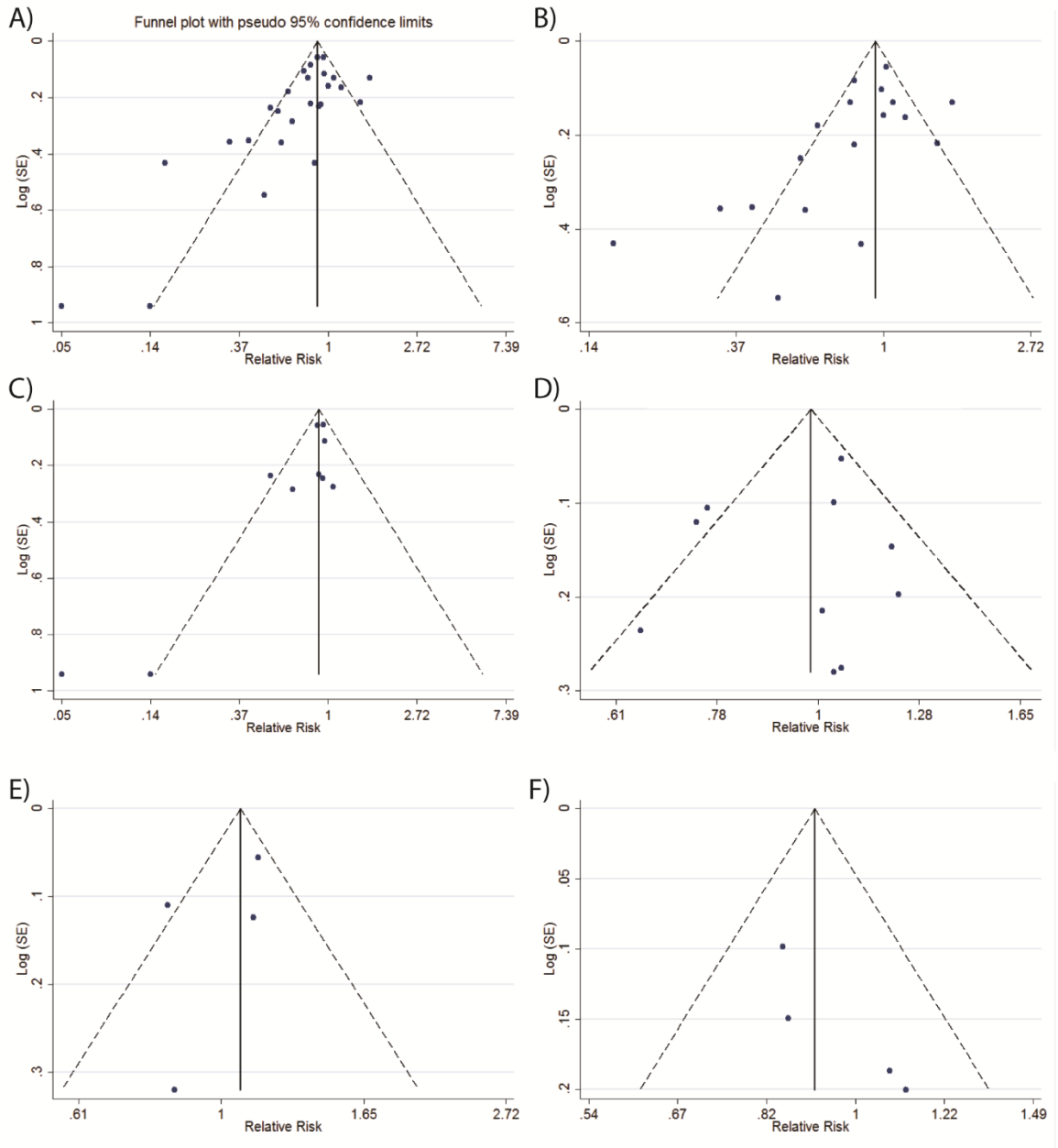
Source Author, Year	Foods included in tomato foods category
Darlington, 2007	Tomato juices, raw tomatoes, and ketchup
Diallo, 2016	Tomato products, specific sources not disclosed
Er, 2014	Tomato juice, tomato sauce, pizza, and baked beans
Hodge, 2004	Tomatoes, pizza, and pasta
Jain, 1999	Tomatoes, tomato soups, and sauces
Jian, 2005	Tomatoes and tomato products, specific sources not disclosed
Kirsh, 2006	Raw tomatoes, canned tomatoes, ketchup, spaghetti, tomato sauce, tomato juice, pizza, lasagna
Kolonel, 2000	Tomatoes, pizza, spaghetti sauce, stews, stir-fries, and others (not disclosed)
Li, 2008	Tomatoes (cooked or raw), specific sources not disclosed
Mills, 1989	Tomatoes, specific sources not disclosed
Norrish, 2000	Tomatoes (cooked or raw), tomato soup or puree, tomato juice, tomato sauce, ketchup, tomato-based pasta dishes
Salem, 2011	Tomatoes (fresh, extract, and dressing)
Sonoda, 2004	Tomatoes, specific sources not disclosed
Stram, 2006	Tomatoes (cooked or raw), tomato sauce, tomato juice, tomato soup, spaghetti, ravioli, lasagna, pizza, and Mexican/Spanish rice
Subahir, 2009	Tomatoes, specific sources not disclosed
Takachi, 2010	Tomatoes, tomato juice, ketchup
Villeneuve, 1999	Tomatoes or tomato juice
Vlajinac, 2010	Tomato foods, specific sources not disclosed

Figure B.1 Forest plot for cumulative meta-analysis of highest vs lowest quantile of overall tomato consumption on PCa risk.



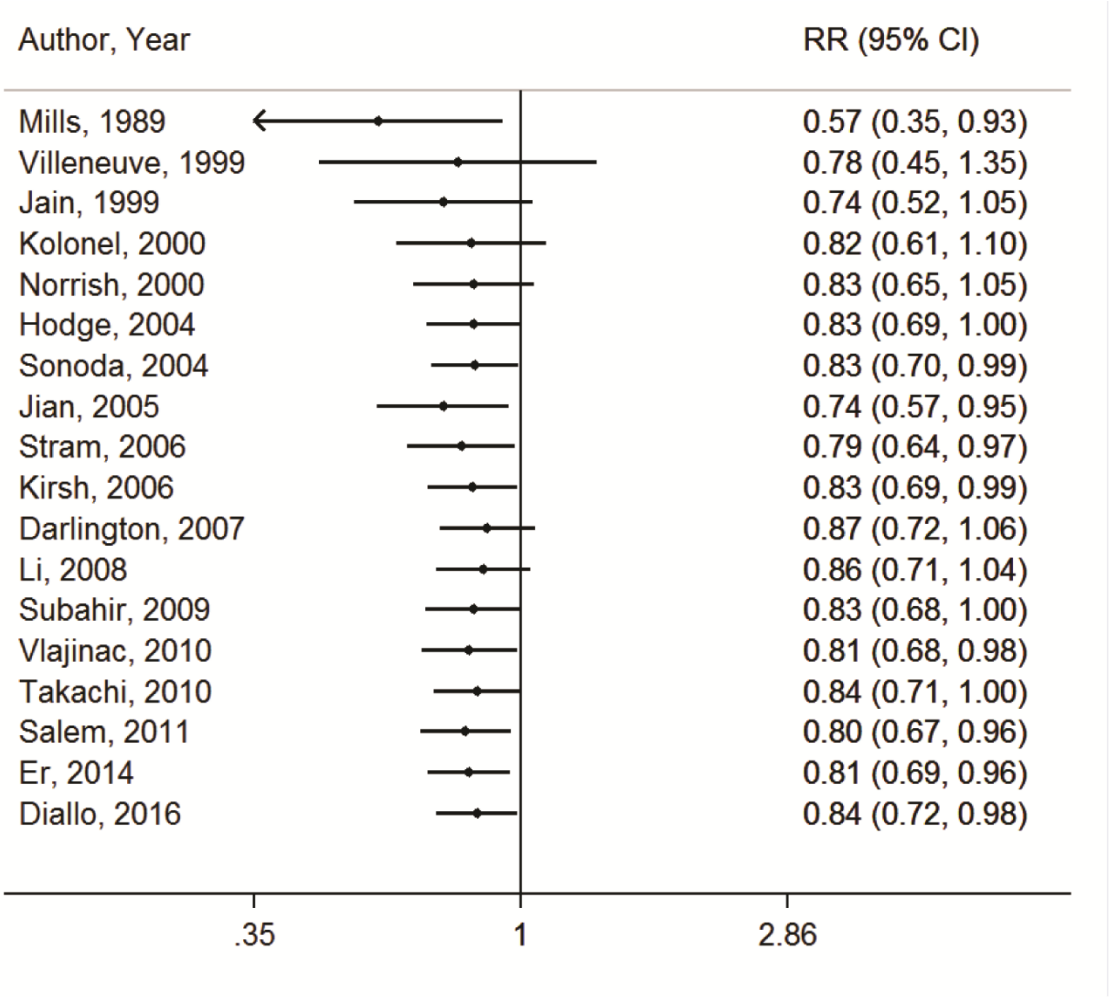
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure B.2. Funnel plot for publication bias among studies investigating tomatoes and PCa risk.



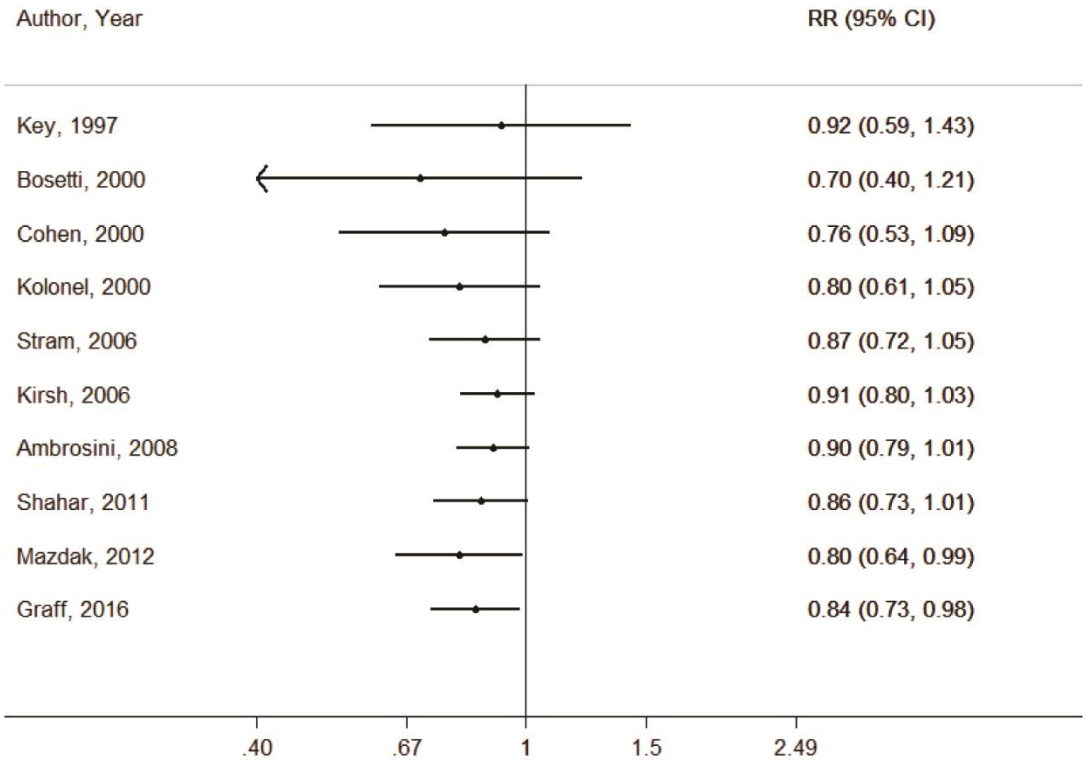
(A) total tomato consumption, (B) tomato foods; (C) cooked tomatoes and sauces, (D) raw tomatoes, (E) pizza and PCa (F) tomato consumption and risk of advanced PCa.

Figure B.3. Forest plot for cumulative meta-analysis of highest vs lowest quantile of consumption of tomato foods on PCa risk.



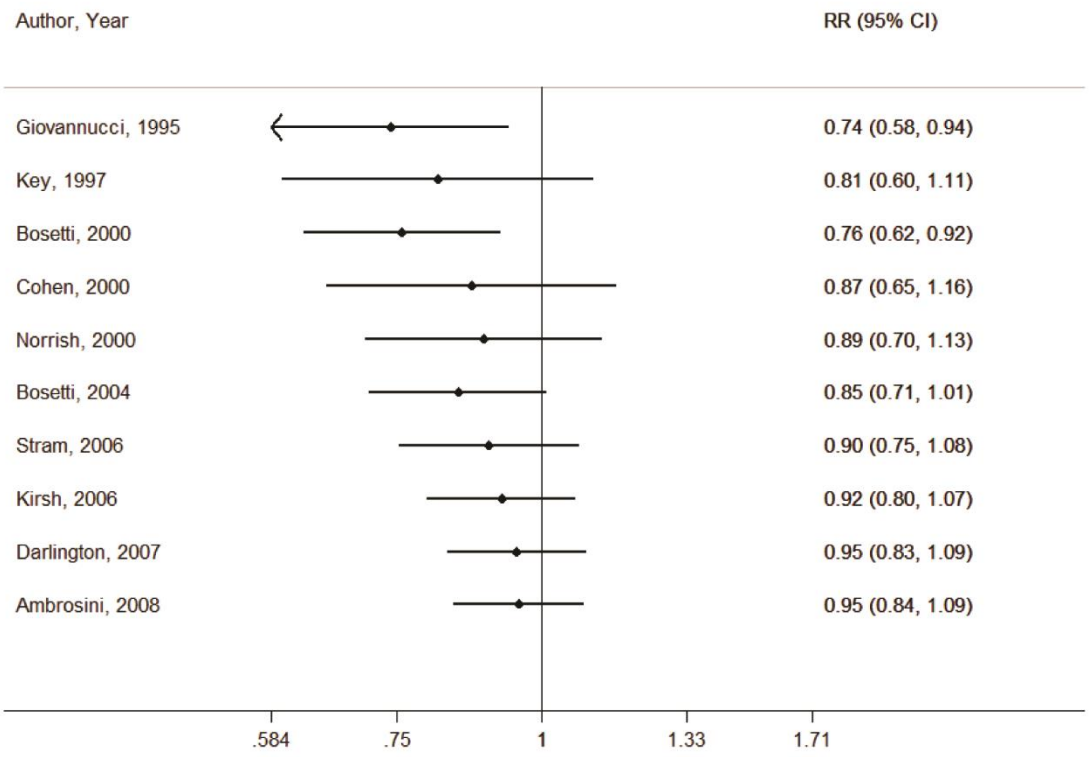
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure B.4. Forest plot for cumulative meta-analysis of highest vs lowest quantile of consumption of cooked tomatoes and sauces on PCa risk.



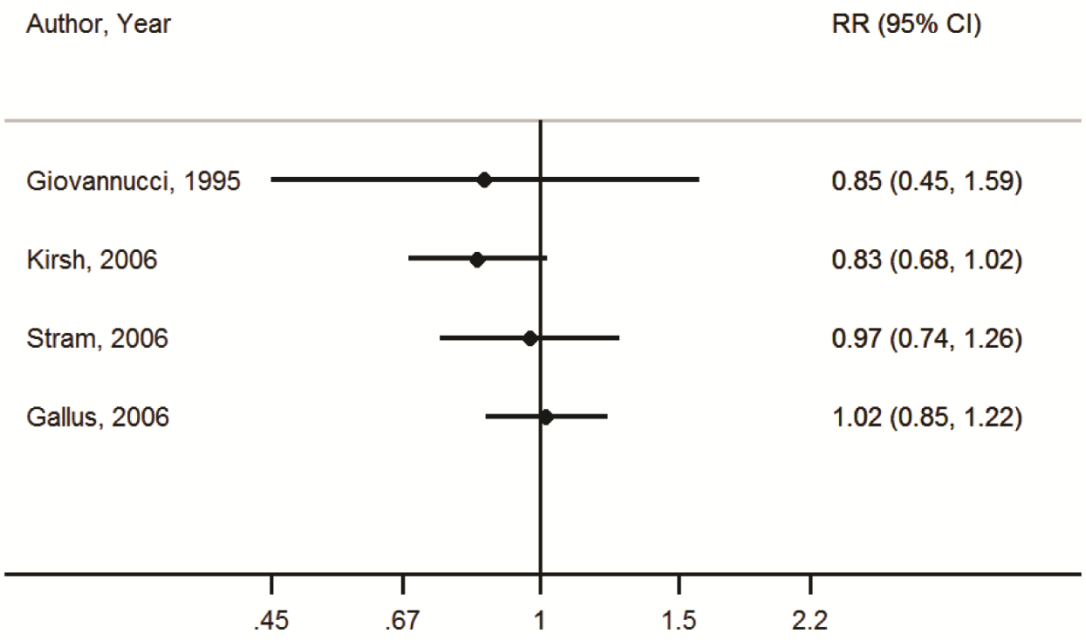
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure B.5. Forest plot for cumulative meta-analysis of highest vs lowest quantile of consumption of raw tomatoes on PCa risk.



This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure B.6. Forest plot for cumulative meta-analysis of highest vs lowest quantile of consumption of pizza on PCa risk.



This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure B.7. Dose-response analysis of tomato consumption and PCa risk by study type.

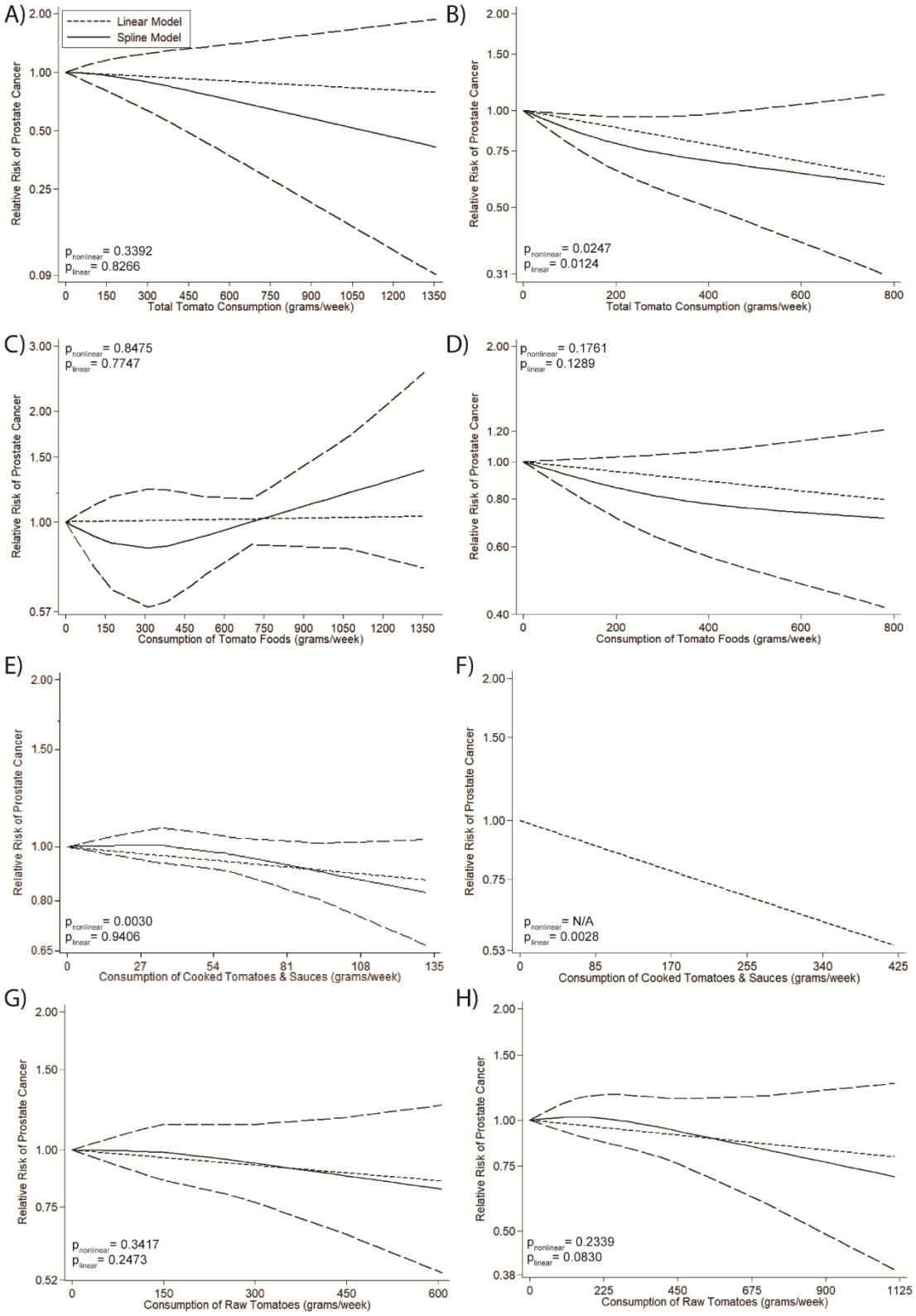
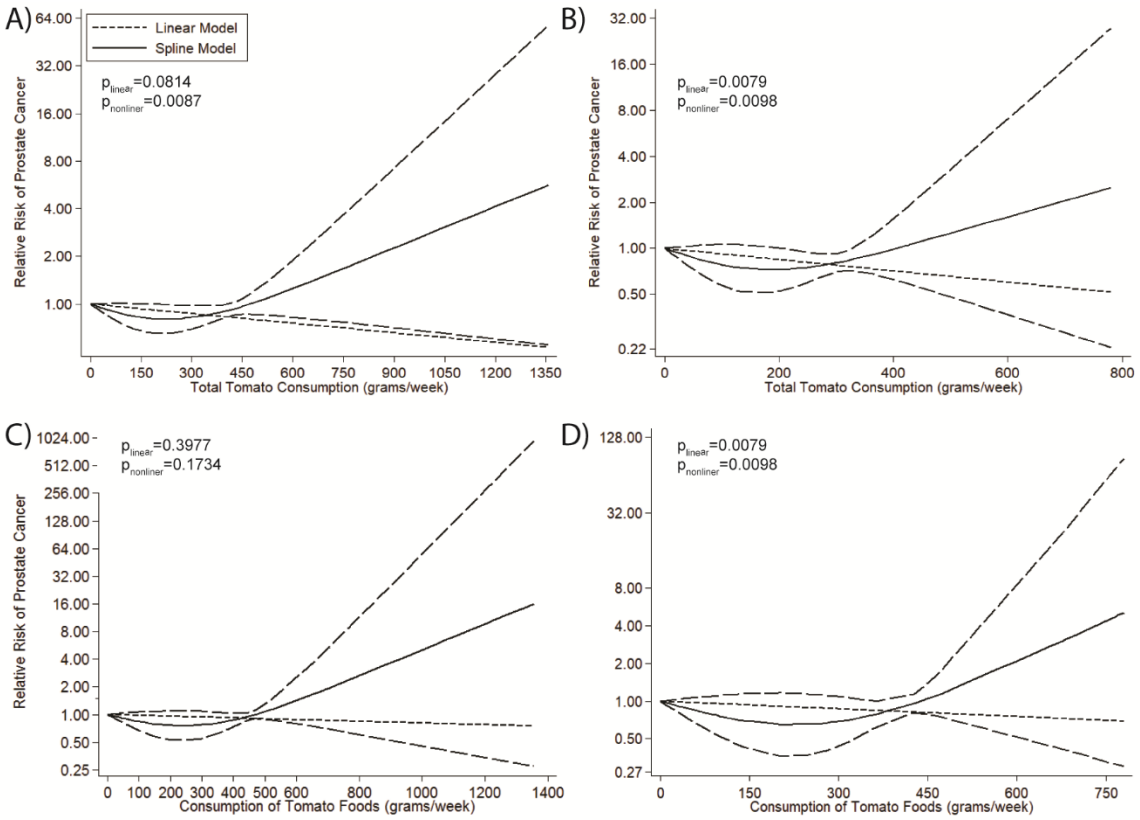


Figure B.7 Caption: The solid black line is the nonlinear model curve for published relative risk and the two dotted lines are the corresponding 95% confidence intervals. The short-dashed line is the linear model curve for the published relative risks (RR); (A) cohort studies for total tomato consumption and risk of PCa; (B) case-control studies for total tomato consumption and risk of PCa; (C) cohort studies for tomato foods and risk of PCa; (D) case-control studies for tomato foods and risk of PCa; (E) cohort studies for cooked tomatoes & sauces and risk of PCa; (F) case-control studies for cooked tomatoes & sauces and risk of PCa; (G) cohort studies for raw tomatoes and risk of PCa; (H) case-control studies for raw tomatoes and risk of PCa.

Figure B.8. Dose-response analysis of tomato consumption and PCa risk before exclusion of one study



The solid black line is the nonlinear model curve for published relative risk and the two dotted lines are the corresponding 95% confidence intervals. The short-dashed line is the linear model curve for the published relative risks (RR); (A) total tomato consumption and risk of PCa; (B) case-control studies for total tomato consumption and risk of PCa; (C) tomato foods and risk of PCa; (D) case-control studies for tomato foods and risk of PCa.

Appendix C: Supplemental figures for Chapter 3

Figure C.1. Funnel plot for publication bias among studies investigating lycopene and PCa risk. (A) dietary lycopene and risk of PCa; (B) circulating lycopene concentrations and risk of PCa; (C) dietary lycopene and risk of advanced PCa; (D) circulating lycopene concentrations and risk of advanced PCa.

Figure C.2 Forest plot for cumulative meta-analysis of highest vs lowest intake of lycopene on PCa risk. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.3. Forest plot for the association of highest vs lowest intake of lycopene on PCa after removing one study. This association was indicated as relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.4. Forest plot for association of highest vs lowest intake of lycopene on PCa risk excluding supplemented studies. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.5. Forest plot for cumulative meta-analysis of highest vs lowest intake of lycopene on advanced PCa risk. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.6. Forest plot for cumulative meta-analysis of highest vs lowest circulating lycopene on PCa risk. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

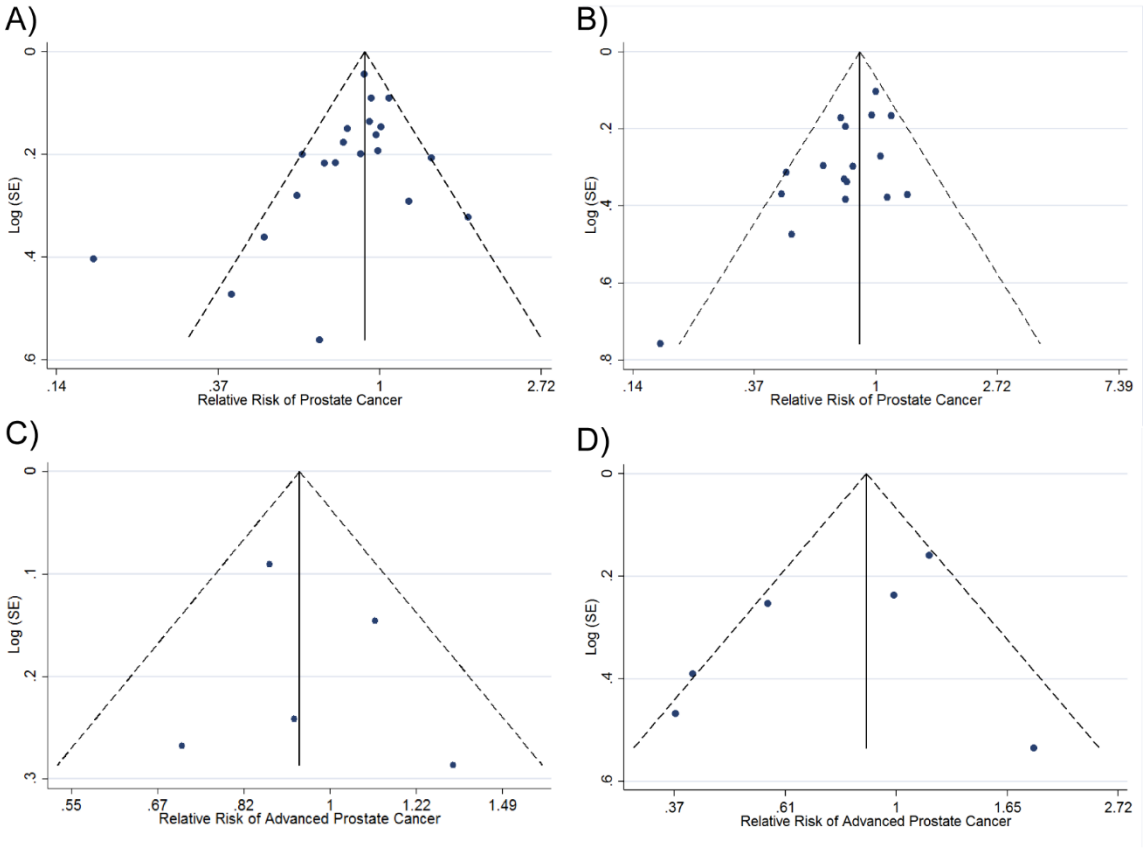
Figure C.7. Forest plot for the association of highest vs lowest circulating lycopene on PCa after removing one study. This association was indicated as relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.8. Forest plot for association of highest vs lowest circulating lycopene on PCa risk excluding supplemented studies. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.9. Forest plot for cumulative meta-analysis of highest vs lowest circulating lycopene on advanced PCa risk. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

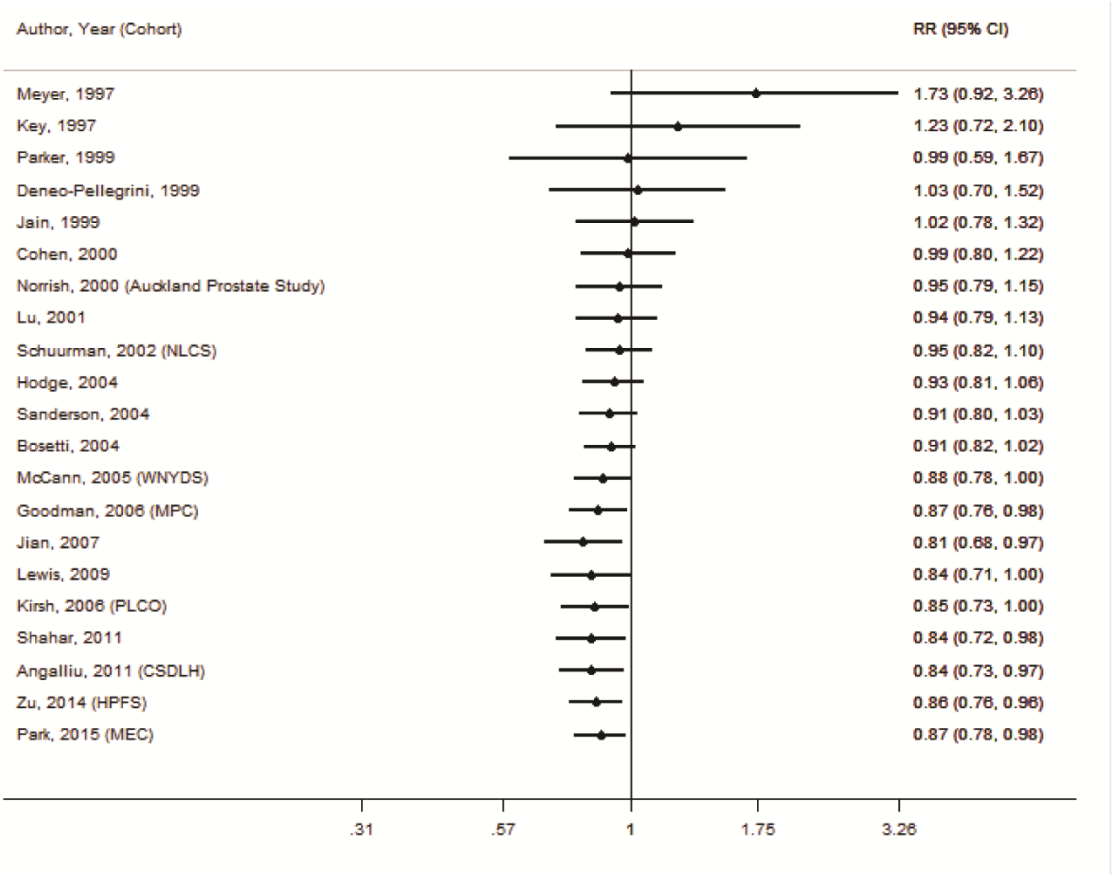
Figure C.10. Forest plot for the association of highest vs. lowest circulating lycopene on advanced PCa risk excluding supplemented studies. This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.1. Funnel plot for publication bias among studies investigating lycopene and PCa risk.



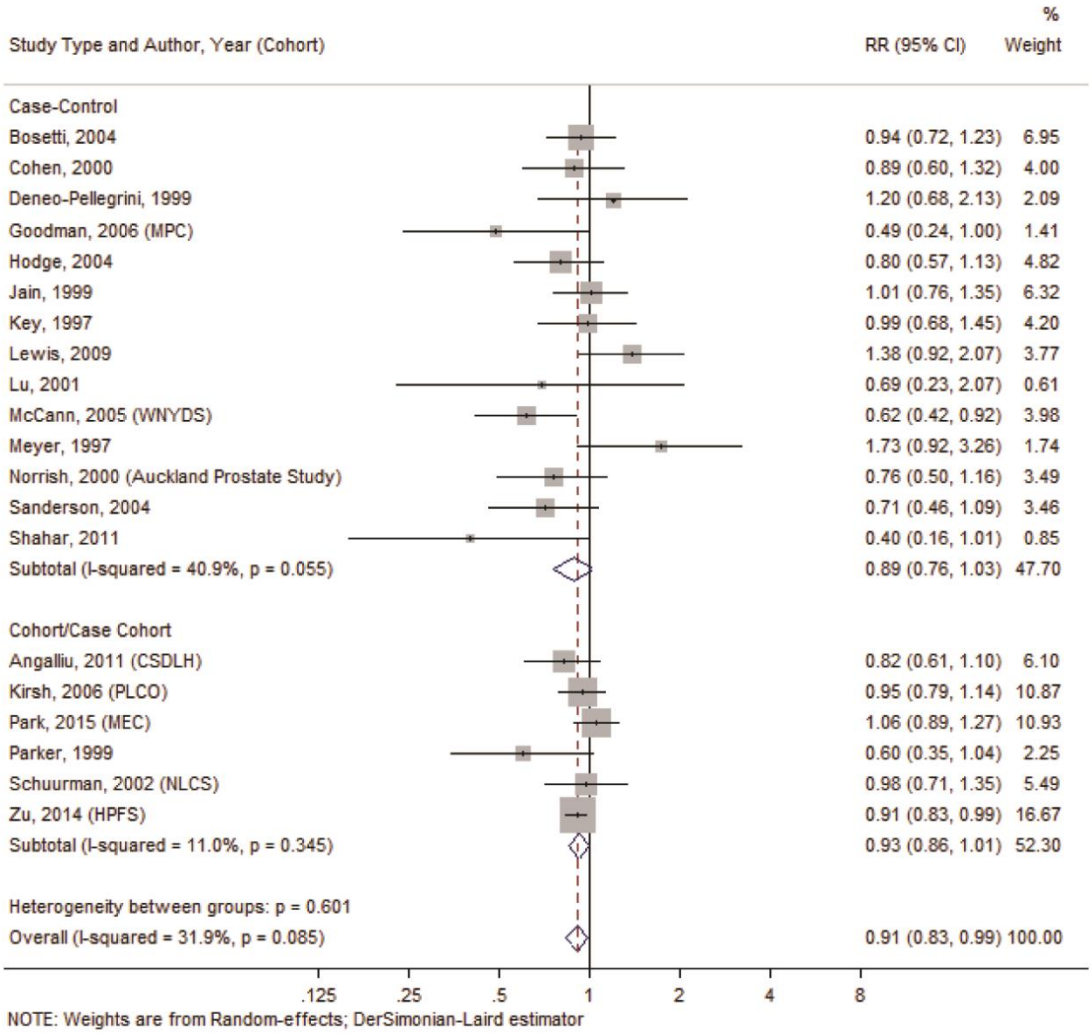
(A) dietary lycopene and risk of PCa; (B) circulating lycopene concentrations and risk of PCa; (C) dietary lycopene and risk of advanced PCa; (D) circulating lycopene concentrations and risk of advanced PCa.

Figure C.2 Forest plot for cumulative meta-analysis of highest vs lowest intake of lycopene on PCa risk.



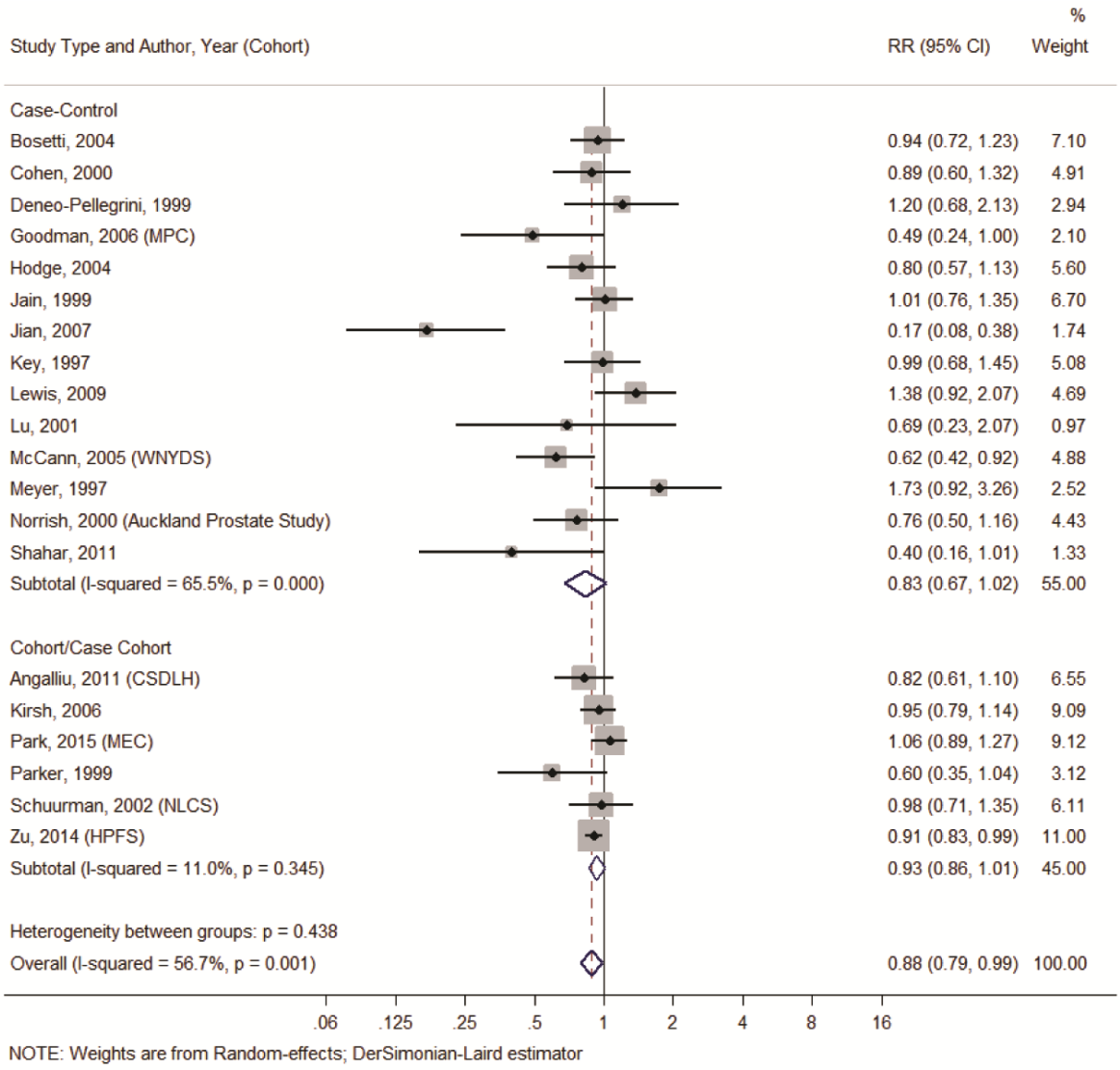
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.3. Forest plot for the association of highest vs lowest intake of lycopene on PCa after removing one study



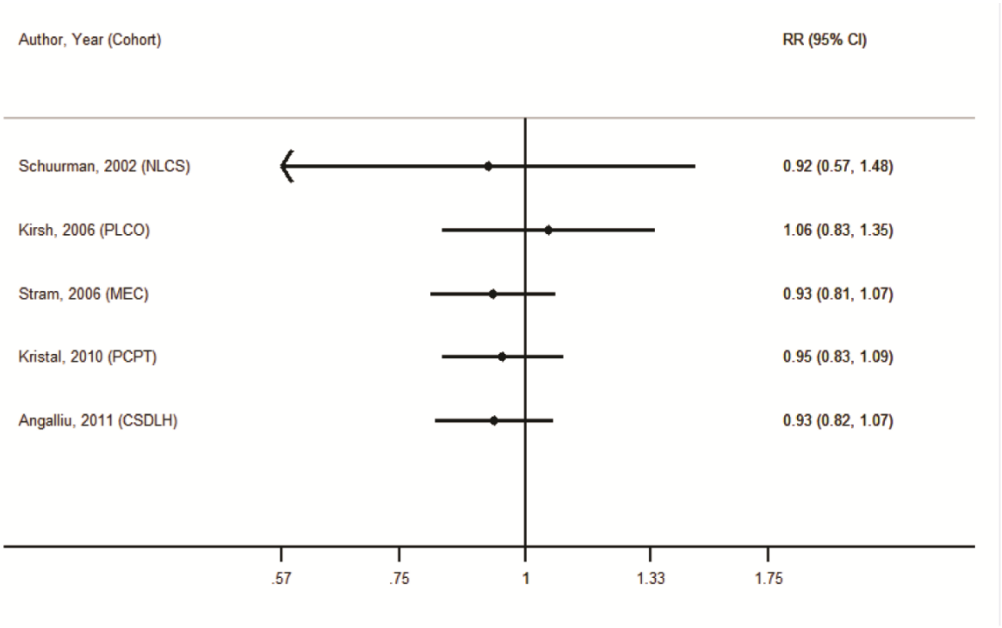
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.4. Forest plot for association of highest vs lowest intake of lycopene on PCa risk excluding supplemented studies.



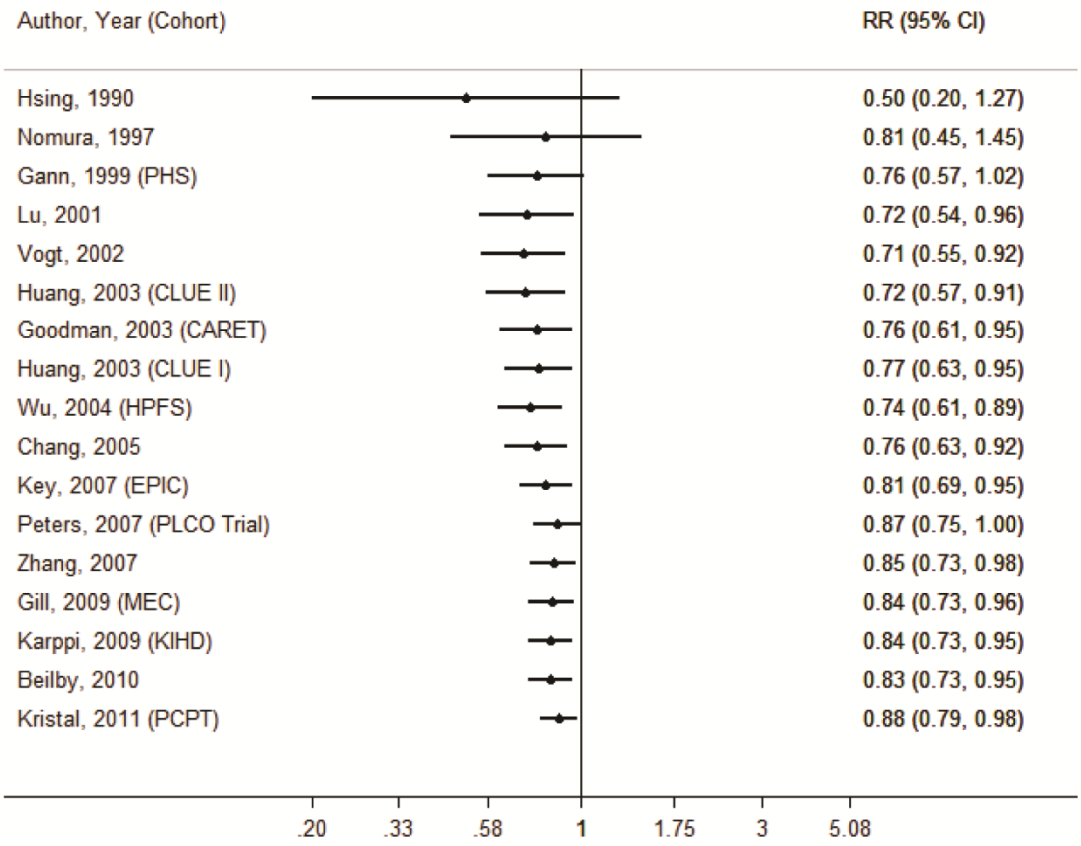
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.5. Forest plot for cumulative meta-analysis of highest vs lowest intake of lycopene on advanced PCa risk



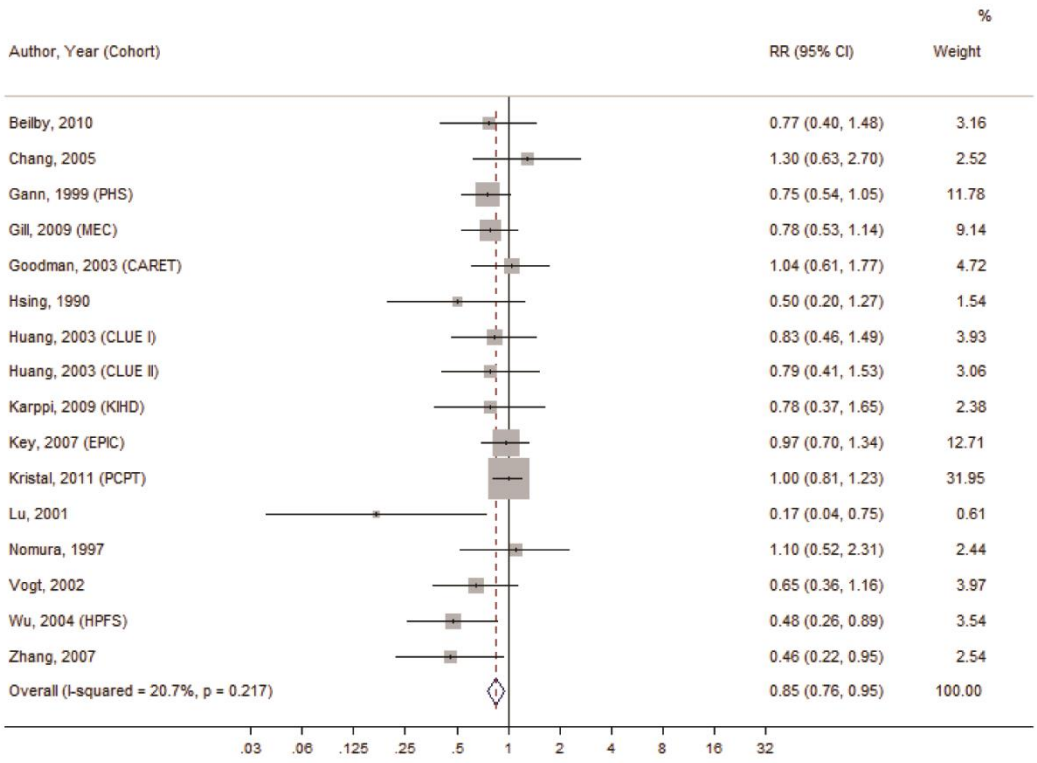
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.6. Forest plot for cumulative meta-analysis of highest vs lowest circulating lycopene on PCa risk.



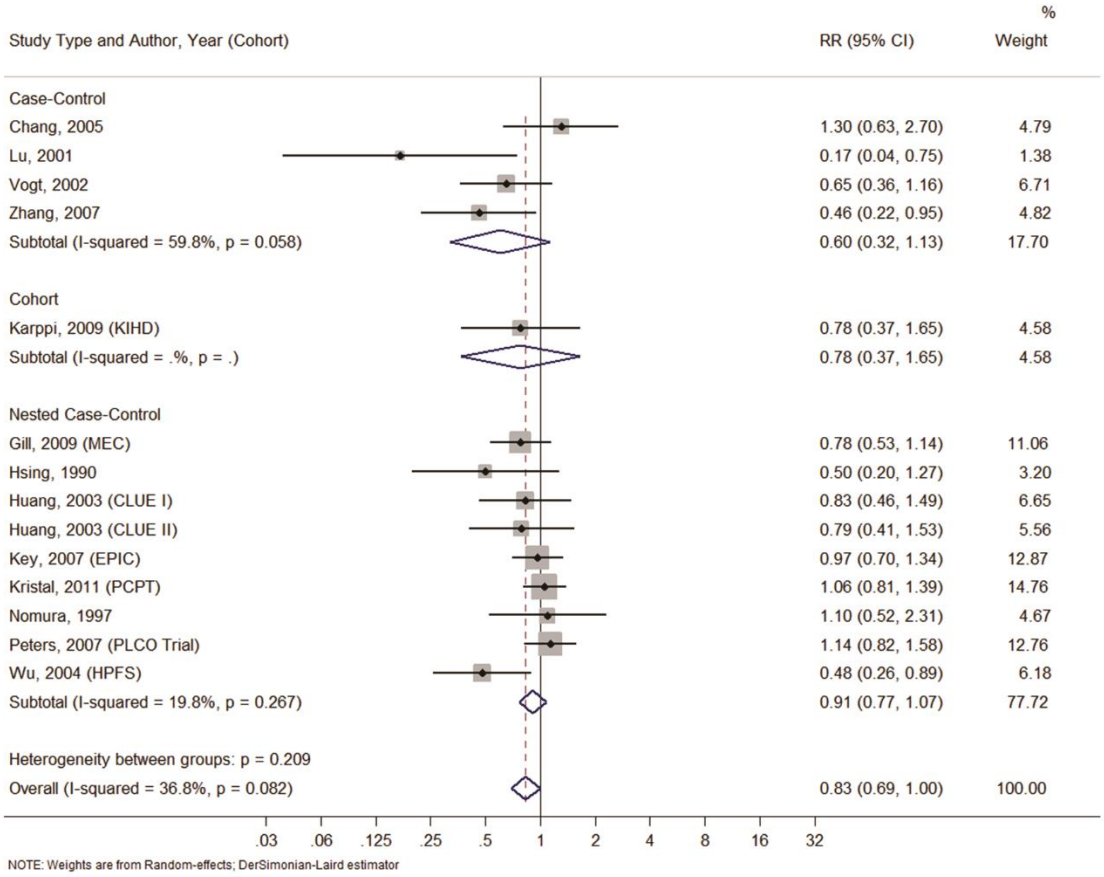
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.7 Forest plot for the association of highest vs lowest circulating lycopene on PCa after removing one study



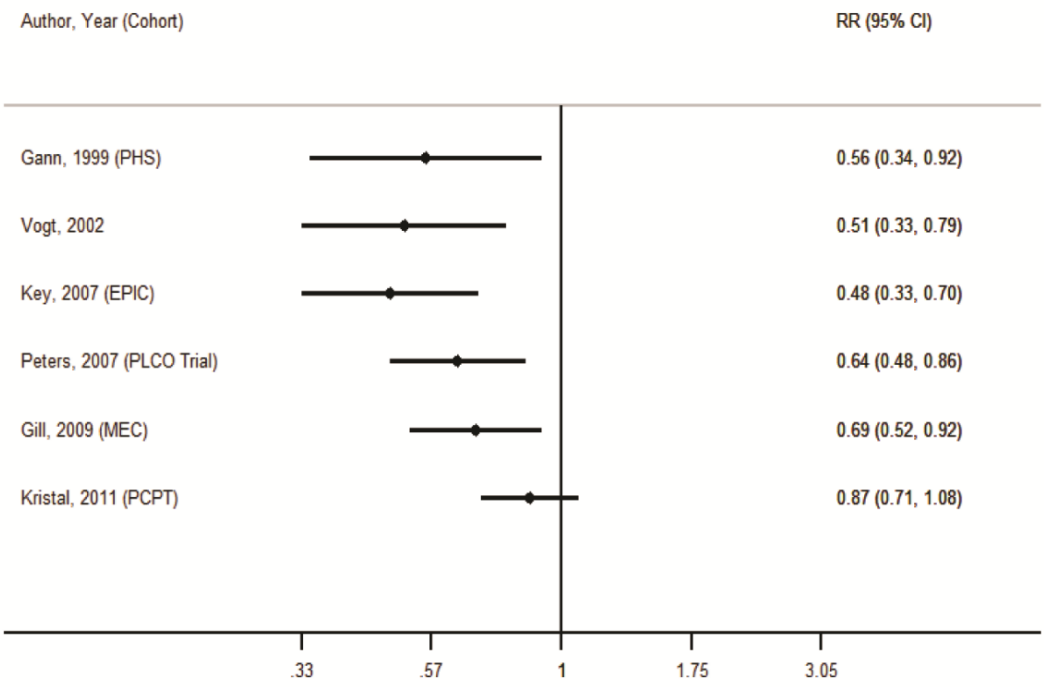
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.8 Forest plot for association of highest vs lowest circulating lycopene on PCa risk excluding supplemented studies.



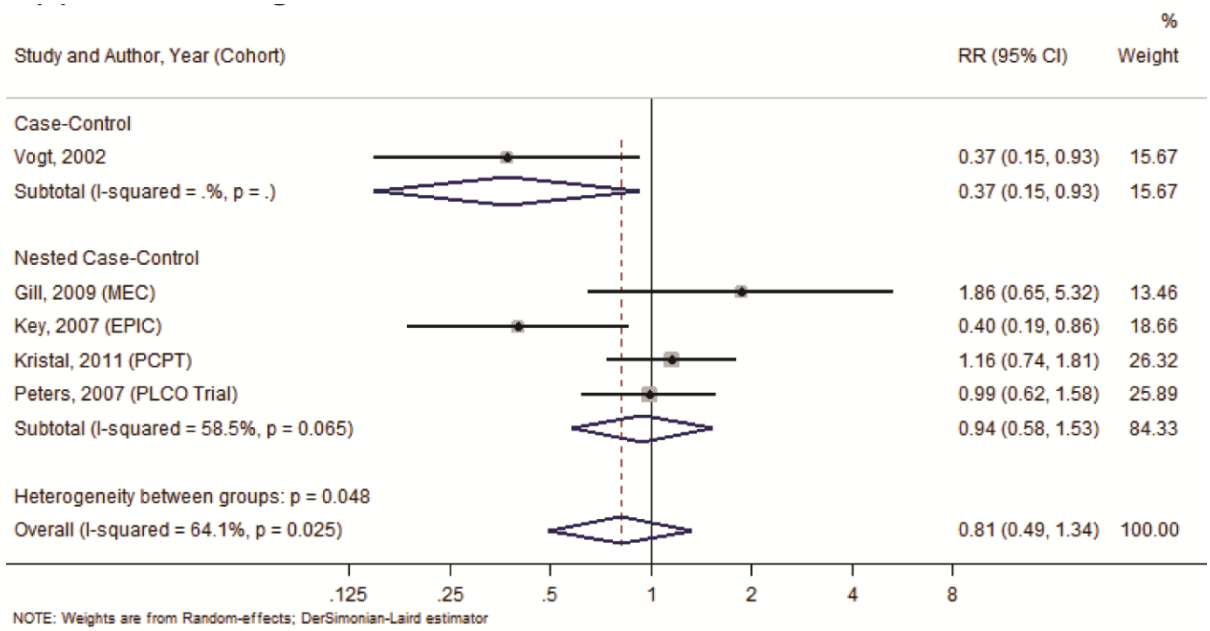
This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.9 Forest plot for cumulative meta-analysis of highest vs lowest circulating lycopene on advanced PCa risk.



This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Figure C.10 Forest plot for the association of highest vs. lowest circulating lycopene on advanced PCa risk excluding supplemented studies.



This association was indicated as a relative risk (RR) estimate with the corresponding 95% confidence interval (CI).

Appendix D: Supplemental table and figures for Chapter 4

Table D.1. Organ weights at necropsy in castrated TRAMP mice.

Figure D.1. Body Weights of CRPC animals

A, Study 1 body weights at euthanasia; individual points indicate individual mice, while the bars represent mean ± SEM. B, Study 1 average weekly body weight; data are represented as mean ± SEM. C, Study 2 body weights; individual points indicate individual mice, while the bars represent mean ± SEM. D, Study 2 average weekly body weight; data are represented as mean ± SEM.

Figure D.2. Individual CRPC tumor growth curves

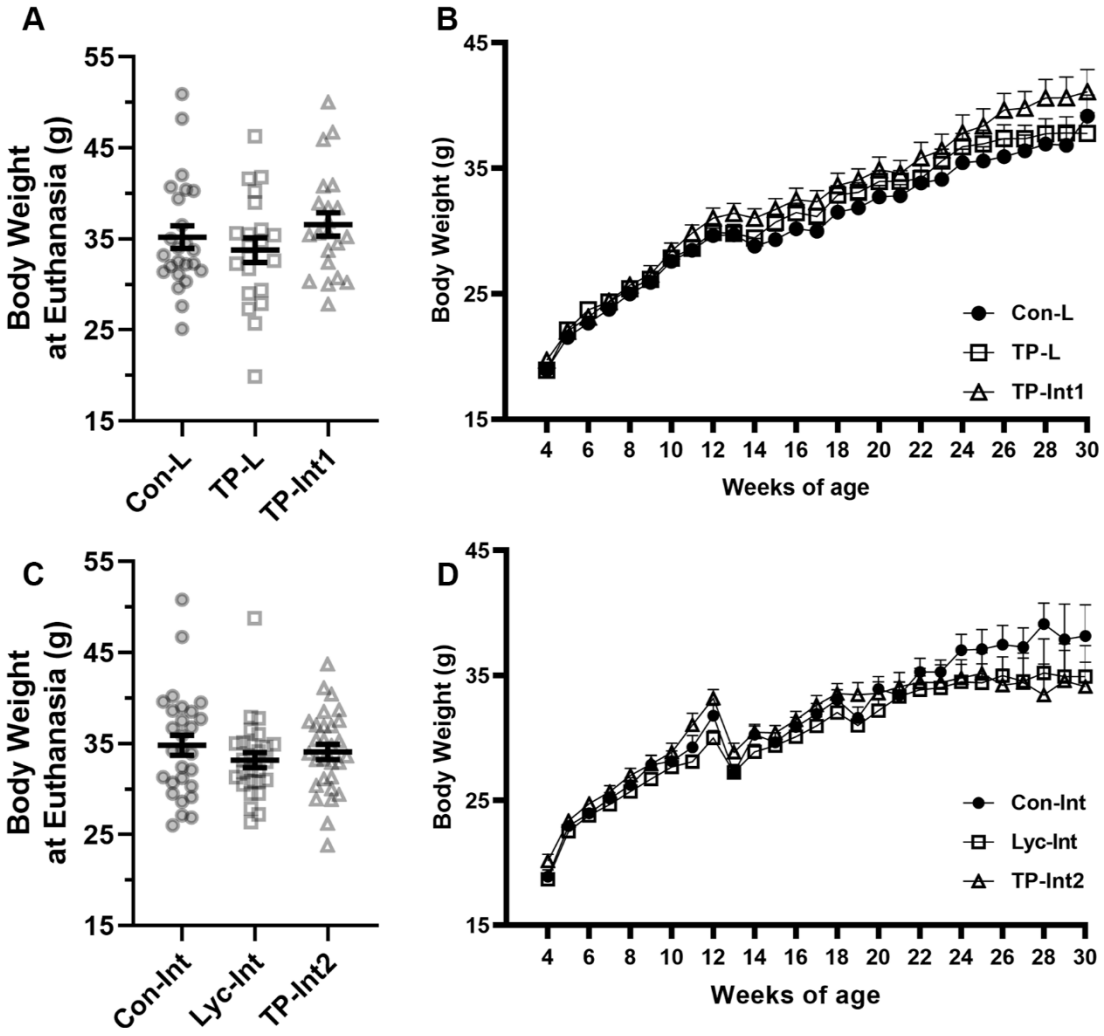
Each line represents the *in vivo* growth of an individual mouse's tumor from detection (week 0) through 4 weeks post-detection. Study 1, top row; Study 2, bottom row. Lines which end before the 4th week are due to animals that died prior to completing all tumor scans. Insets are provided to magnify volumes ≤ 1500 mm^3

Table D.1. Organ weights at necropsy in castrated TRAMP mice.

Study 1	**Con-L**	**TP-L**	**TP-Int1**	**p-value**
Prostate Tumor (g)	2.9 ± 0.5	2.6 ± 0.6	1.9 ± 0.5	0.173
Prostate[2] (mg)	27.8 ± 3.2	19.6 ± 1.5	22.8 ± 2.2	0.248
Anterior	18.6 ±2.6	12.8 ± 1.1	15.6 ± 1.8	0.311
Dorsolateral	13.0 ± 2.8	6.3 ± 0.7	6.9 ± 0.8	0.076
Ventral	4.4 ±0.4	4.6 ± 0.8	7.0 ± 0.8	0.680
Seminal Vesicles (mg)	13.8 ±0.8	14.2 ± 1.4	17.6 ± 2.7	0.341
Liver (g)	1.44 ±0.08	1.22 ± 0.07	1.33 ± 0.06	0.071
Lungs (g)	0.24 ±0.04	0.21 ± 0.03	0.17 ± 0.01	0.059
Epididymal Adipose Tissue (g)	1.75 ± 0.21	1.87 ± 0.21	2.4 ± 0.25	0.137
Study 2	**Con-Int**	**Lyc-Int**	**TP-Int2**	**p-value**
Prostate Tumor (g)	1.9 ± 0.3	2.4 ± 0.5	2.1 ± 0.3	0.658
Prostate[2] (mg)	11.6 ± 2.6	7.6 ± 1.3	6.3 ±1.1	0.182
Anterior	8.7 ± 2.6	6.5 ± 1.6	4.0 ± 0.7	0.136
Dorsolateral	4.5 ± 0.8	4.4± 1.3	4.3 ± 0.8	0.760
Ventral	3.6 ± 0.8	2.6 ± 0.3	3.7 ± 1.0	0.553
Seminal Vesicles (mg)	27.0 ± 3.4	38.2 ± 13.9	27.3 ± 8.4	0.465
Liver (g)	1.24 ± 0.12	1.15 ± 0.05	1.20 ± 0.03	0.087
Lungs (g)	0.19 ± 0.02	0.26 ± 0.04	0.21 ± 0.02	0.208
Epididymal Adipose Tissue (g)	2.03 ± 0.23	1.65 ±0.17	1.72 ± 0.19	0.366

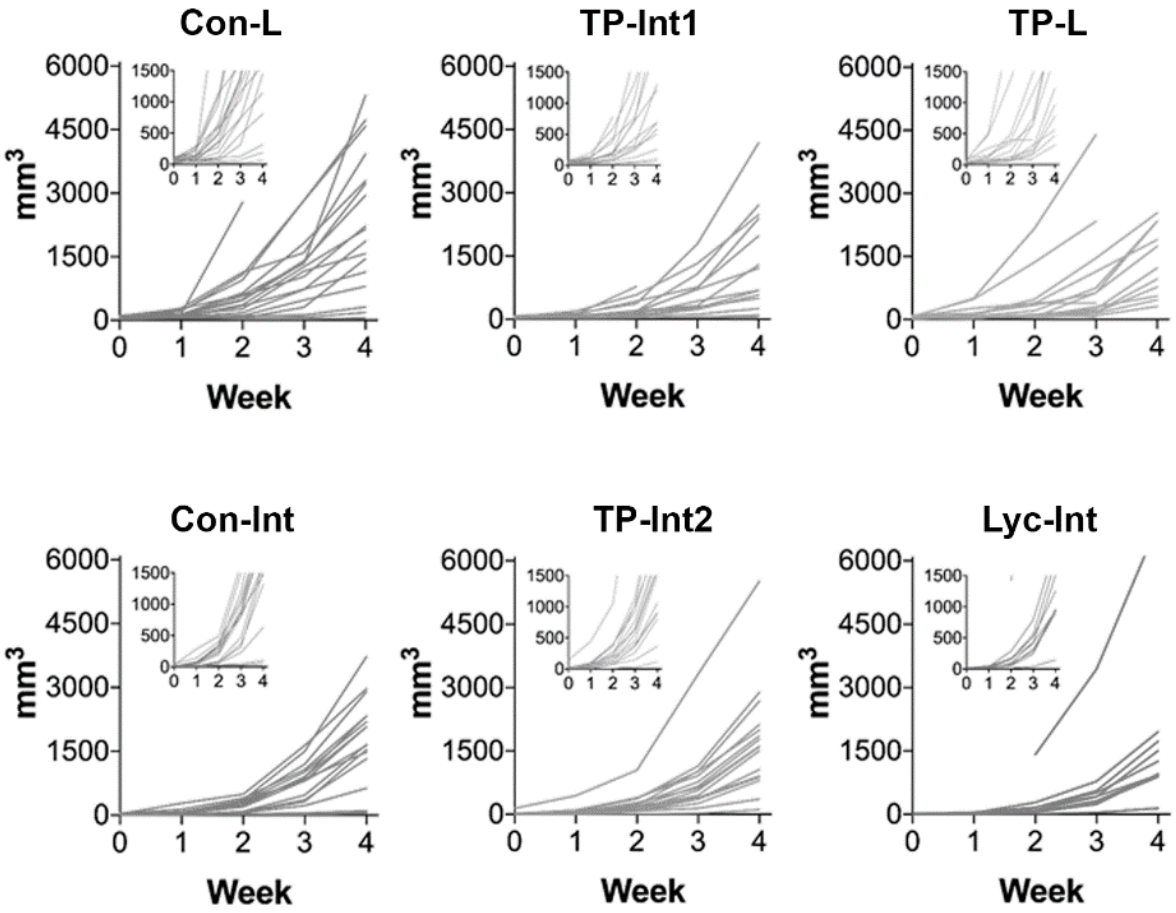
[1] There were no statistically significant differences between treatments for any organ at necropsy by ANOVA. Values are represented as mean ± SEM. In Study 1, n=26-28 per group (Con-L, n=28; TP-L, n=26; TP-Int1, n=26). In Study 2, n=27-29 per group (Con-Int, n=29; TP-Int2, n=29; Lyc-Int, n=27).
[2] Represents the combined weight of all lobes of prostate found at necropsy.

Figure D.1. Body Weights of castrated TRAMP mice fed tomato (TP) and lycopene (Lyc) containing diets



A, Study 1 mean body weight of TRAMP mice at euthanasia; B, Study 1 mean weekly body weight; C, Study 2 mean body weight of TRAMP mice at euthanasia; D, Study 2 mean weekly body weight. Data are represented as mean ± SEM. Individual points indicate individual mice. In Study 1, n=26-28 per group (Con-L, n=28; TP-L, n=26; TP-Int1, n=26). In Study 2, n=27-29 per group (Con-Int, n=29; TP-Int2, n=29; Lyc-Int, n=27).

Figure D.2. Individual CRPC tumor growth curves



Each line represents the *in vivo* growth of an individual mouse's tumor from detection (week 0) through 4 weeks post-detection. Study 1, top row; Study 2, bottom row. Lines which end before the 4th week are due to animals that died prior to completing all tumor scans. Insets are provided to magnify volumes ≤ 1500 mm^3.

Appendix E: Supplemental tables and figures for Chapter 5

Figure E.1. Dose of radiation delivered

Dose of radiation received. Values are represented as mean ± SEM. The dotted line represents the targeted dose of 7.5 gy. * means differ ($p<0.0001$, t-test)

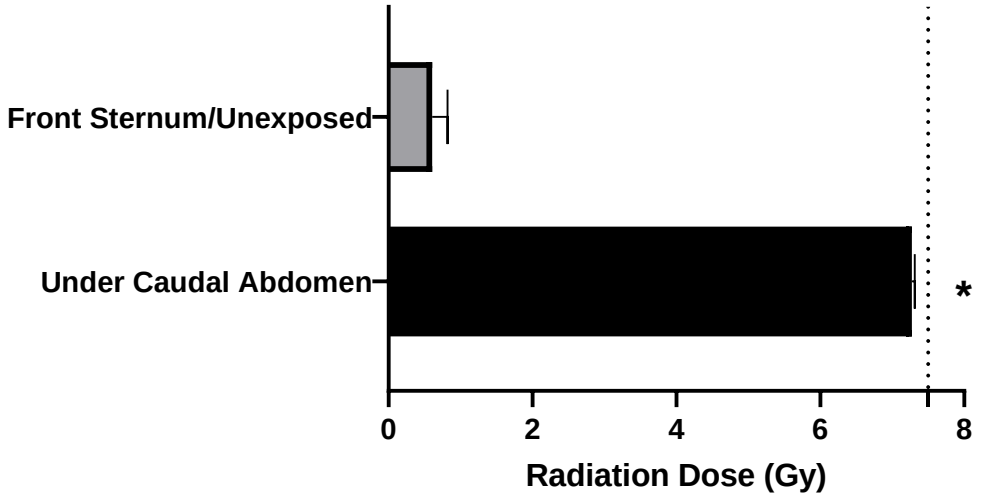
Figure E.2. Body weights of TRAMP mice

A, Pilot study body weights at euthanasia, bars represented as mean ± SEM. B, Pilot study mean weekly body weight; data are represented as mean ± SEM. C, Diet study body weights; values are represented as mean ± SEM. D, Diet study mean weekly body weight; data are represented as mean ± SEM.

Table E.1. Composition of experimental diets.

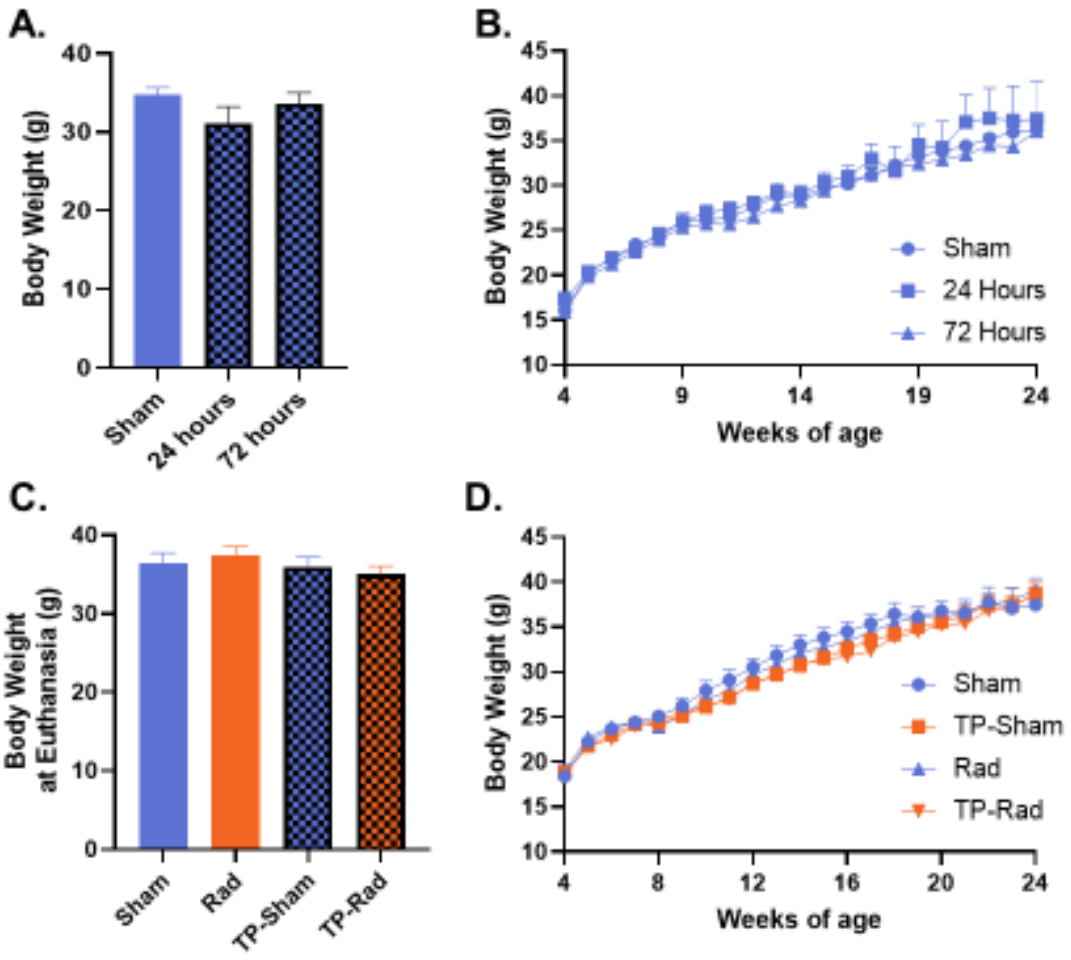
Table E.2. Organ weights at necropsy in TRAMP mice.

Figure E.1. Dose of radiation delivered



Values are represented as mean ± SEM (n=3 per group). The dotted line represents the targeted dose of 7.5 gy. * means differ ($p<0.0001$, t-test)

Figure E.2 Body weights of TRAMP mice



A, Pilot study body weights at euthanasia, bars represented as mean ± SEM (n=6/group). B, Pilot study mean weekly body weight (n=6 per group); data are represented as mean ± SEM. C, Diet study body weights; values are represented as mean ± SEM (Sham, n=16; TP-Sham, n=20; Rad, n=19; TP-Rad, n=18). D, Diet study mean weekly body weight; data are represented as mean ± SEM (Sham, n=16; TP-Sham, n=20; Rad, n=19; TP-Rad, n=18).

Table E.1 Composition of experimental diets.

	Control	10% Tomato
Cornstarch	390	363
Maltodextrin	130	105
Sucrose	98	97
Casein	196	177
Cellulose	49	41
AIN-93G mineral mix	34	34
AIN-93G vitamin mix	10	10
L-Cystine	3.0	3.0
Choline bitartrate	2.5	2.5
Soybean oil	70	68
Lyophilized tomato paste	0	100
10% Lycopene beadlets	0	0
Water	18	0
kcal/g diet[1]	3.9	3.8

[1]Calculated based on results from proximate analysis.

Table E.2 Organ weights at necropsy

Pilot Study	**Sham**	**24 Hours**	**72 Hours**	**p-value**
Prostate Tumor (g)	0.88 ± 0.32	0.53 ± 0.18	0.28 ± 0.05	n.a.
Prostate[2] (mg)	312 ± 110	108 ± 23	257 ± 53	0.06
Anterior	137 ± 25	87 ± 16	153 ± 28	0.17
Dorsolateral	169 ± 134	25 ± 9	89 ± 22	0.19
Ventral	94 ± 48	14 ± 1	20 ± 3	n.a.
Seminal Vesicles (g)	0.97 ± 0.26	0.76 ± 0.08	1.32 ± 0.24	0.06
Liver (g)	1.34 ± 0.08[a]	1.08 ± 0.07[b]	1.12 ± 0.04[ab]	**0.03**
Lungs (g)	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.00	0.69
Epididymal Adipose (g)	1.03 ± 0.11	0.88 ± 0.16	1.01 ± 0.08	0.61
Heart (mg)	141 ± 5	140 ± 6	140 ± 5	0.76
Testes (mg)	231 ± 7[a]	213 ± 5[ab]	207 ± 5[b]	**<0.01**
Spleen (mg)	83 ± 4[a]	40 ± 4[b]	40 ± 4[b]	**<0.0001**

Diet Study	**Sham**	**TP-Sham**	**Rad**	**TP-Rad**	
Prostate Tumor (g)	1.65 ± 0.30	1.73 ± 0.49	1.24 ± 0.30	1.68 ± 0.27	0.58
Prostate[1] (mg)	197 ± 37	203 ± 28	247 ± 24	196 ± 33	0.28
Anterior	136 ± 24	119 ± 14	151 ± 15	124 ± 22	0.56
Dorsolateral	58 ± 11	78 ± 13	73 ± 9	56 ± 9	0.34
Ventral	40 ± 6	36 ± 3	33 ± 3	29 ± 4	0.23
Seminal Vesicles (g)	0.82 ± 0.13	0.78 ± 0.10	0.96 ± 0.11	0.89 ± 0.12	0.65
Liver (g)	1.38 ± 0.06	1.39 ± 0.07	1.37 ± 0.07	1.32 ± 0.04	0.76
Lungs (g)	0.21 ± 0.06	0.15 ± 0.01	0.20 ± 0.04	0.15 ± 0.00	0.53
Epididymal Adipose (g)	1.25 ± 0.11	1.42 ± 0.12	1.31 ± 0.11	1.33 ± 0.10	0.76
Heart (mg)	151 ± 5	158 ± 4	160 ± 4	156 ± 3	0.46
Testes (mg)	239 ± 4	238 ± 4	234 ± 4	231 ± 3	0.13
Spleen (mg)	88 ± 10[a]	64 ± 16[a]	82 ± 6[b]	44 ± 2[b]	**<0.0001**

Values are represented as mean ± SEM. In the pilot study, n=6 per group (Sham, n=6; 24-Hours, n=6; 72-Hours, n=6). In the Diet Study, n=16-20 per group (Sham, n=16; TP-Sham, n=20; Rad, n=19; TP-Rad, n=18). Bolded entries signify a main treatment (radiation) effect of an ANOVA. n.a. represents not analyzed due to a low number of samples (n=2) in one of the groups.
[1]Represents the combined weight of all lobes of prostate found at necropsy.