A Meta-Analysis of Dietary Carotenoids and Prostate Cancer Incidence

Thesis · April 2014
DOI: 10.13140/RG.2.1.5074.7761

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A Meta-Analysis of Dietary Carotenoids and Prostate Cancer Incidence

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A Thesis Submitted in Partial Fulfilment of Module PG4902 for the degree of B.Sc. in Human Health & Disease

Contents

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Acknowledgements

There are a number of people I would like to thank at MEB who facilitated me in carrying out this project. Firstly, I would like to thank Dr. Katarina Bälter for allowing me to complete my degree project at Karolinska Institutet. You, and everybody else in the research group made me feel very welcome and made my time there very enjoyable. To my co-supervisors Jennifer Protudjer and Arvid Sjölander – thank you for all your help and invaluable knowledge and guidance throughout this project. And also Camilla Wiklund for always brightening up the office.

My family have been incredibly supportive throughout my time in Stockholm, particularly Paul Leacy and Sinead Mac. Thanks to mam, dad and the kids for always being there and encouraging me to do my best. My friends have also been incredibly helpful and enthusiastic while I've been away, and they never fail to put a smile on my face.

To those mentioned and everybody else who has helped me through these last three months – tack så mycket!

The doctor of the future will give no medicine, but will interest her or his patients in the care of the human frame, in a proper diet, and in the cause and prevention of disease.

Thomas Edison, US inventor (1847 - 1931)

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1. Abstract

Background: Prostate cancer is one of the most common cancers in the world. However, disparities in incidence rates worldwide have suggested that lifestyle factors, particularly diet, may play a role in its development. Carotenoids have exhibited multiple anti-cancer effects, and increased intakes of high-carotenoid foods have been shown to be protective against prostate cancer in epidemiological investigations. The aim of this project was to complete a meta-analysis of dietary intake of four carotenoids – α-carotene, β-cryptoxanthin, lutein, and zeaxanthin – to determine their role in prostate cancer incidence.

Methods: A PubMed literature search and a systematic review of the literature was performed to identify studies measuring carotenoid intake and prostate cancer risk. Estimates of OR or HR for highest versus lowest categories of intake were pooled for each individual carotenoid for case-control/NCC studies and cohort/case-cohort studies, respectively. Tests for heterogeneity and publication bias were also carried out.

Results: A total of sixteen published articles were included in the analysis. A significantly reduced risk of prostate cancer was found for higher intakes of each of the four carotenoids in case-control/NCC studies, but not for cohort/case-cohort studies. Pooled ORs for lutein (0.76, 95% CI = 0.60-0.97, p = 0.03) and lutein & zeaxanthin (0.82, 95% CI = 0.75-0.89, p = 0.00) showed the strongest risk reductions, while α-carotene (OR = 0.92, 95% CI = 0.84-1.00, p = 0.04) and β-cryptoxanthin (OR = 0.91, 95% CI = 0.83-0.99, p = 0.03) showed more modest protective effects. Cohort/case-cohort studies also expressed reduced risks for higher intakes (lutein showed no association; HR = 1.00, 95% CI = 0.91-1.10, p = 0.97), though these results were not statistically significant. No publication bias was detected, though there was significant heterogeneity between included studies.

Conclusion: There appears to be an inverse association for intake of α-carotene, β-cryptoxanthin, lutein, and zeaxanthin and prostate cancer. Increased intakes of high carotenoid foods may be protective against prostate cancer development.

2. Background

2.1. Prostate Cancer Epidemiology

Prostate cancer accounts for 15% of all cancer diagnosed in men, with over 1.1 million cases in 2012[1]. Prostate cancer is the most common cancer in Europe, with an estimated incidence rate of 96.0 per 100,000[2]. Sweden and Ireland have the 3rd and 4th highest rates in Europe, with 175.2 and 168.7 per 100,000 respectively. These high incidence rates have been attributed to an increased prevalence of prostate-specific antigen (PSA) testing, leading to higher diagnoses of non-fatal prostate cancers[3]. Mortality rates have remained relatively low (307,000 deaths worldwide in 2012), despite a high prevalence of latent prostate cancer at all ages (Figure 1). Autopsy studies have suggested that the majority of men over 85 years of age have some degree of histological prostate cancer[4].

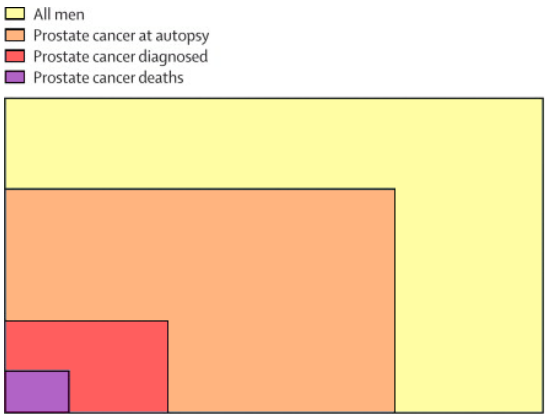

Figure 1 - Relation Between Prevalence of Prostate Cancer at Autopsy, Clinically Diagnosed Prostate Cancer, and Prostate Cancer Deaths. Adapted from Damber and Aus[9]

Cancer of the prostate generally occurs in men over the age of 65, and there is an increased risk for men whose family have history of the disease[5]. Initiation of prostate cancer involves downregulation of multiple molecular signalling pathways, though androgen receptor signalling is key for progression to aggressive forms[6]. A number of genetic factors have been identified, and epigenetic mechanisms are emerging as a candidate for novel treatments[7]. Benign prostatic hyperplasia (BPH) is another common condition of the prostate which shares many pathophysiological traits with prostate cancer[8].

Despite being the second most common cancer among men, there exists substantial worldwide variance in prostate cancer incidence, with developed countries accounting for almost 70% of cases[1]. The highest incidence rates are reported in Australia & New Zealand, North America, and Northern and Western Europe, with South-East & South-Central Asian regions reporting the lowest rates. Rates in Asia are almost six times lower than their 'Western' counterparts[10]. Disparities also exist between prostate cancer incidence and mortality worldwide (Figure 2). Furthermore migrants from low- to high-risk regions experience increased incidence and mortality from prostate cancer within two generations[11]. Taken together, these trends are highly suggestive of the influence of environmental factors upon prostate cancer risk.

As the old adage goes; prevention is better than cure. The key aim of cancer chemoprevention studies of dietary constituents is to identify active ingredients and explicate their underlying mechanisms. This can aid in designing strategies for intervention trials, and ultimately educate people on the best ways

to avoid illness and improve their overall health. Prostate cancer is an ideal candidate for chemoprevention studies. The high incidence rates and long latency period mean there is a large therapeutic window for dietary intervention treatment. Identifying conclusive dietary factors which are protective or damaging to prostate cancer development will create a simple, effective, and low-cost method for reducing disease prevalence worldwide.

A recent British study determined that almost 43% of cancers are attributed to lifestyle factors. In the context of prostate cancer, smoking[13] and obesity[14] are associated with higher risks of fatal prostate cancer (30% and 15%, respectively). A recent study carried out by this research group at Karolinska[15] showed that higher levels of physical activity were associated with decreased rates of prostate cancer mortality, with a hazard ratio of 0.62 (95% CI = 0.41-0.94) for men who walked/bicycled 20-60 minutes per day compared to men in the lowest category.

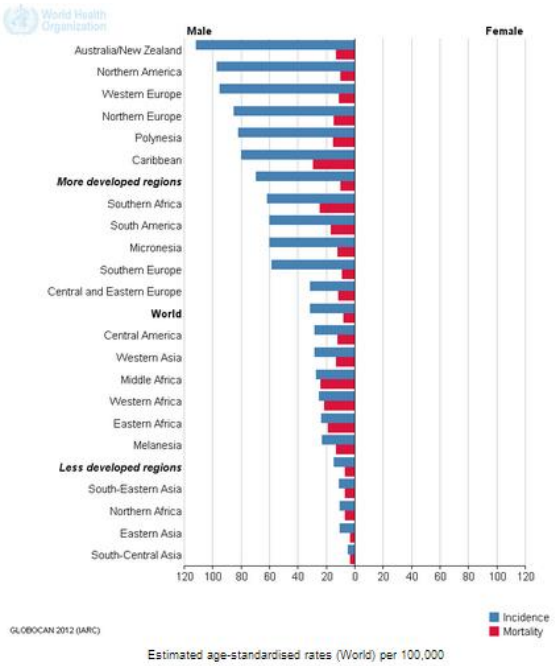

Figure 2 - Disparities in Prostate Cancer Incidence Worldwide[1]

2.2. Diet and Prostate Cancer

One lifestyle factor that has received considerable focus in relation to prostate cancer is diet. There have been a number of epidemiological investigations into the influences of different foods and prostate cancer risk. Although an analysis of the EPIC cohort[16] (European Prospective Investigation into Cancer and Nutrition) found no association between fruit and vegetable intake and prostate cancer incidence, analysis of specific vegetables and groups have found significant associations.

Several analyses of different fruits and vegetable classes have been completed, but there is considerable variance in how studies classify vegetables into specific groups. Notable studies involve the analysis of effects of legume and cruciferous vegetable intake. Legumes have been widely examined due to the influence of soy foods and soy isoflavones in prostate cancer, which have demonstrated significant protective effects[17]. Cruciferous vegetables, particularly those from the Brassica family, have a significant protective effect against prostate cancer in both epidemiologic and laboratory studies[18]. In a recent meta-analysis by Liu et al[19], high consumers of cruciferous vegetables had a relative risk of 0.90 (95% CI = 0.85-0.96) for prostate cancer compared to low consumers. Though analysis of the effects of vegetables can be beneficial, these analyses do not reflect the independent influences of foods (or nutrients) within their groupings.

There have been a number of meta-analyses of different dietary factors and their effect on prostate cancer incidence (Appendix 1). Fish[20], cruciferous vegetables[19], coffee[21], and total soy food[17] have all been shown to significantly decrease prostate cancer risk, while dairy products[22] and total fat[23] increase risk. Similarly, food constituents have been examined to determine their influence on the disease. Daidzein and genistein, two common isoflavones found in soy, were found to decrease prostate cancer incidence when consumed at higher amounts[17].

Of the completed meta-analyses that reached statistical significance, many of them have examined foods which are high in carotenoids. Carotenoids have exhibited multiple protective effects against cancer (see sections 2.3-2.7), and are commonly consumed by many cultures. The most recent meta-analysis on this topic related to carrot intake and prostate cancer risk[24]. The analysis of 10 studies showed a significantly decreased risk of prostate cancer (OR = 0.82, 95% CI = 0.70-0.97) for men with high compared to low carrot intakes. This study also found a dose-response association between carrot consumption and reduced risk of prostate cancer. An increase of one serving of carrots per week yielded a risk estimate of 0.95 (95% CI = 0.90-0.99), and for each 10g per day increase this estimate was 0.96 (95% CI = 0.94-0.99). Carrots are particularly high in α-carotene, and many other foods which have been shown to be protective against prostate cancer contain high concentrations of carotenoids.

2.3. Carotenoids

Carotenoids are fat-soluble organic pigments that are found in the chloroplasts and chromoplasts of plants. The name "carotene" actually comes from the Latin *carota*, meaning 'carrot'. Carotenoids are responsible for the bright, orange-hued colours of many foods, and some of them are used as food colourings.

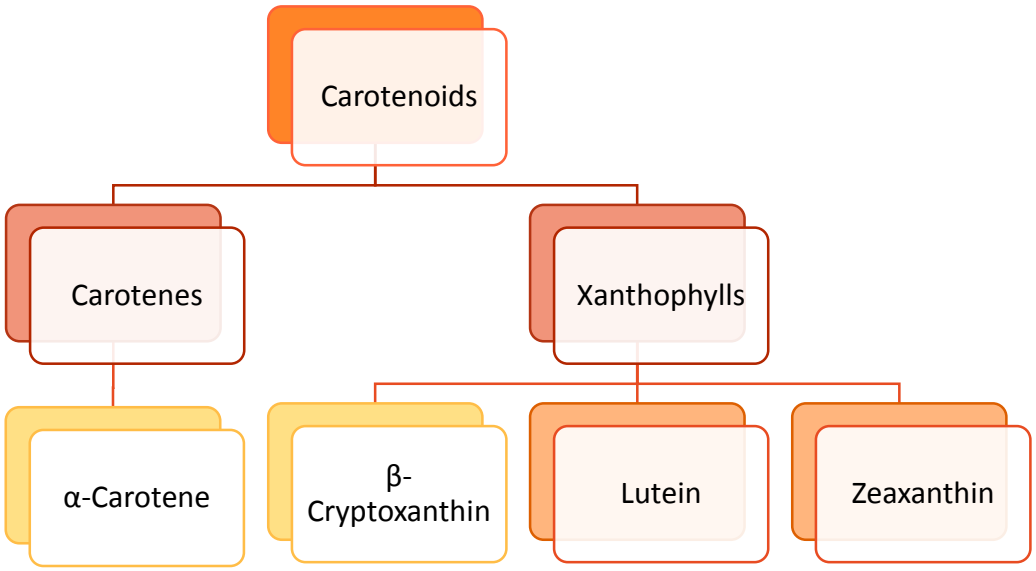

Figure 3 - Classification of Carotenoids. Those shown in yellow have vitamin A activity.

Over 700 carotenoids have been identified to date, though only a fraction of these are present in human diets[25]. They are split into two classes (Figure 3); xanthophylls (which contain oxygen) and carotenes (which do not contain oxygen). Carotenoids are biosynthesised from common precursors (geranylgeranyl pyrophosphate) and a number of enzymes facilitate their production. Humans cannot synthesise carotenoids themselves and must acquire them entirely from dietary sources. People with diets rich in carotenoids are at a lower risk for cardiovascular and ocular diseases, as well as many cancers[26]. The health benefits of carotenoids may be in part explained by their relationship with vitamin A. Vitamin A is essential for a variety of biological processes, including organogenesis, immune competence, tissue differentiation, and the visual cycle[27]. Some carotenoids have provitamin A activity, and their relationships with vitamin A may synergistically contribute to their overall health benefits.

The most prevalent dietary carotenoids are α-carotene, β-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin[28]. However, because they are not classified as essential nutrients, values for recommended daily intake have not been established[29]. Carotenoids are found in a wide variety of foods, most commonly fruits and vegetables. Top dietary sources for α-carotene, β-cryptoxanthin, and lutein & zeaxanthin are shown in Tables 1-3 respectively. Hardin et al[30] investigated the influence of high intakes of fruits and vegetables rich in carotenoids (as well as other dietary components) on prostate cancer. High carotenoid fruits encompassed apricots, orange juice, grapefruit juice, peaches, nectarines, plums, cantaloupe, orange melon, mango, oranges, grapefruits, and tangerines. High carotenoid vegetables included in the analysis were beans, fresh tomatoes, tomato juice, vegetable juice, broccoli, cauliflower, cabbage, Brussels sprouts, green peas, vegetable, minestrone, and tomato soups, carrots, green salad, winter and summer squash, red peppers/chilies, yams/sweet potatoes, spinach, mustard greens, and collards.

Lycopene is the most abundant carotenoid found in blood and has already been extensively studied as a possible chemopreventative tool for prostate cancer. Having demonstrated multiple anti-cancer mechanisms in in-vitro studies[31], investigations into lycopene intake have shown a protective effect against prostate cancer[32] (OR for higher intakes compared to lower = 0.93; 95% CI = 0.86-1.01). High intakes of tomatoes (the most abundant source of lycopene) have also led to reduced risks compared to lower intakes (OR = 0.81; 95% CI = 0.59-1.10). Another carotenoid that has been highly investigated in the context of cancer is β-carotene. A large RCT found that β-carotene supplementation led to an increased rate of cancer at several sites, including a 23% increase in incidence and 15% increase in mortality from prostate cancer[33]. The World Cancer Research Fund Report investigated the effect of serum, dietary, and supplemental β-carotene on prostate cancer, and determined that neither β-carotene nor foods containing it are likely to have a substantial effect on the risk of prostate cancer[34].

Table 1 – Top Food Sources of α-Carotene, taken from USDA Food Composition Database[35]

Description	µg/100 g
Carrot, dehydrated	14,251
Peppers, sweet, red, freeze-dried	6,931
Pumpkin, canned, without salt	4,795
Pumpkin, canned, with salt	4,795
Carrot juice, canned	4,342
Pumpkin, raw	4,016
Carrots, cooked, boiled, drained, without salt	3,776
Carrots, cooked, boiled, drained, with salt	3,776
Carrots, baby, raw	3,767
Babyfood, carrots and beef, strained	3,716
Carrots, frozen, cooked, boiled, drained, without salt	3,716
Carrots, frozen, cooked, boiled, drained, with salt	3,716
Carrots, raw	3,477
Babyfood, carrots, toddler	3,340
Carrots, frozen, unprepared	2,958
Soup, cream of vegetable, dry, powder	2,820
Carrots, canned, regular pack, drained solids	2,743
Carrots, canned, no salt added, solids and liquids	2,743
Carrots, canned, no salt added, drained solids	2,743
Pumpkin, cooked, boiled, drained, without salt	2,715
Pumpkin, cooked, boiled, drained, with salt	2,715
Carrots, canned, regular pack, solids and liquids	2,692
Babyfood, vegetables, carrots, junior	2,682
Vegetables, mixed, canned, drained solids	2,636

2.4. α-Carotene

α-carotene is the second most common form of carotene after β-carotene. The two differ only in structure by the position of a double bond (and consequentially, a hydrogen atom) in the cyclic group at one end. As it contains a retinyl group (a β-ionone ring which allows an isoprenoid ring to attach), α-carotene has a small degree of provitamin A activity. Serum α-carotene concentrations were inversely associated with all-cause mortality, as well as cardiovascular disease, cancer, and all other causes[36]. α-carotene was also found to inhibit proliferation of endometrial, mammary, and lung human cancer cells in culture[37].

The major dietary sources of α-carotene are from yellow/orange vegetables (carrots, sweet potatoes, winter squash) and dark green vegetables (broccoli, green beans, green peas, spinach, turnip greens, collards, leaf lettuce, avocado). As shown in Table 1, carrots are the top source of α-carotene, and it is also found in high amounts in other yellow-orange vegetables such as peppers and pumpkin.

2.5. β-Cryptoxanthin

β-cryptoxanthin is a xanthophyll, related in structure to β-carotene with only an additional hydroxyl group. Because it also contains an ionone group, it can be converted to retinol to allow provitamin A activity in humans. β-cryptoxanthin has exhibited protective effects against free radical damage in cell culture, and stimulation of DNA repair. Results of studies of blood β-cryptoxanthin and prostate cancer have been contradictory. Some studies indicate a decreased risk with higher blood levels[39, 40], while others show an increase in incidence[41, 42]. The top dietary sources of β-cryptoxanthin are from fruits – tangerines, mangoes oranges and peaches, though spearmint and cilantro (coriander) also contain high levels. Table 2 contains the top food sources in descending order.

2.6. Lutein

Lutein is a xanthophyll with no provitamin A activity. In plants it modulates light energy, and in humans can act as an antioxidant for blue light absorption in the eye. In cell studies lutein demonstrated selective inhibition of malignant prostate cancer cells (AT3) over their benign counterparts (DTE)[43]. 42% of cancerous cells were inhibited after 4 days of culture in 2.0μM of lutein. The most substantial health benefits of lutein however are in the eyes, where higher intakes[44] and supplementation[45] have been shown to improve ocular condition. Although there are no recommended dietary intake guidelines for lutein, positive effects (in the context of decreased risk of age-related macular degeneration) have been seen at dietary intake levels of 6-10mg/day[46].

Dietary sources of lutein & zeaxanthin are shown in Table 3, with high contents found in many green leafy vegetables, cornmeal, beans, oranges and kiwi fruit. Lutein is approved for use as an additive in the EU (E number E161b)[47], and is commonly used in chicken feed to improve the colour of egg yolks, and chicken skin and fat. Rohrmann et al[48] showed that, compared to low intakes (0.2 servings/day), those who consumed 1.4 servings of lutein rich food (cooked/raw spinach, kale, broccoli, Brussels sprouts, celery, peas, and yellow squash) per day had a decreased risk of incident BPH (OR = 0.83; 95% CI = 0.75-0.92; p value for trend = 0.0004).

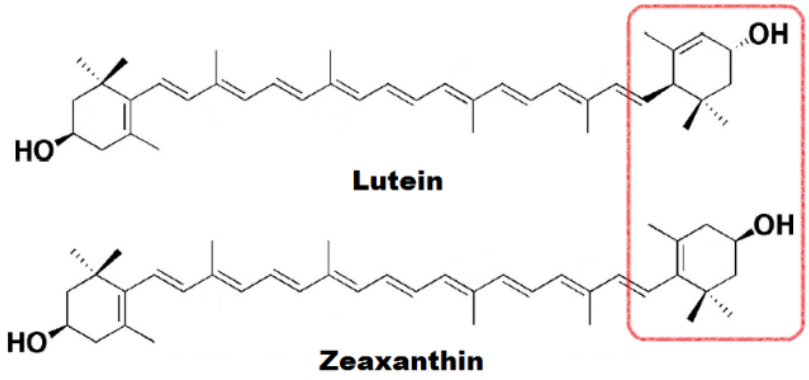

Figure 4 - Differences in Structure of Lutein and Zeaxanthin, Adapted from Abdel-Aal et al[49]

2.7. Zeaxanthin

Zeaxanthin is a xanthophyll, and it is one of the most common carotenoid alcohols found in nature. Like lutein, zeaxanthin regulates light energy in plants, and is found at high concentrations in the retina of human eyes. Higher intakes are associated with a reduced risk of age-related macular degeneration, geographic atrophy, and large or extensive intermediate drusen[44]. Zeaxanthin has been found to induce apoptosis in neuroblastoma cells, without inhibiting lipoxygenase activity or damaging healthy cells[50]. An inverse association between plasma concentrations and prostate cancer was also observed (OR = 0.22; 95% CI = 0.06–0.83; P for trend, 0.0028) when comparing highest with lowest quartiles[51].

The name “zeaxanthin” is derived from *Zea mays*, the trinomial term for maize/corn, and *xanthos*, the Greek word for "yellow". It is the pigment that gives many foods their characteristic colours, including paprika, saffron, corn, and egg yolks. Zeaxanthin contents combined with lutein are shown in Table 3, but top sources of zeaxanthin alone are corn, Japanese persimmons, cornmeal, spinach, turnip greens, collards, lettuce (cos/romaine), kale, tomatoes, tangerines, and oranges[52].

Lutein and zeaxanthin share many functions and characteristics. This is because they are isomers, differing only in the location of the double bond in one of the end rings (Figure 4). Due to their structural similarities, many studies examine lutein and zeaxanthin together rather than individually. No studies were found to examine dietary intake of zeaxanthin alone, and for this reason this project analyses lutein individually, and the combination of lutein and zeaxanthin*.

* “lutein & zeaxanthin” will from this point be used to describe the combined intake of the two carotenoids – “lutein and zeaxanthin combined”

Table 2 – Top Food Sources of β-Cryptoxanthin, taken from USDA Food Composition Database[35]

Description	µg/100 g
Spices, pepper, red or cayenne	6,252
Spices, paprika	6,186
Spices, chili powder	3,490
Squash, winter, butternut, raw	3,471
Squash, winter, butternut, cooked, baked, without salt	3,116
Squash, winter, butternut, cooked, baked, with salt	3,116
Tangerine juice, frozen concentrate, sweetened, undiluted	2,767
Squash, winter, butternut, frozen, unprepared	1,564
Persimmons, Japanese, raw	1,447
Squash, winter, Hubbard, cooked, boiled, mashed, without salt	1,119
Peppers, hot chilli, sun-dried	1,103
Tangerines, (mandarin oranges), canned, juice pack, drained	775
Papayas, raw	589
Tangerines, (mandarin oranges), canned, juice pack	503
Tangerines, (mandarin oranges), canned, light syrup pack	496
Peppers, hot chili, red, canned, excluding seeds, solids and liquids	495
Peppers, sweet, red, raw	490
Rose Hips, wild (Northern Plains Indians)	483
Peppers, sweet, red, cooked, boiled, drained, without salt	460
Peppers, sweet, red, cooked, boiled, drained, with salt	460
Peaches, dried, sulphured, uncooked	444
Tangerines, (mandarin oranges), raw	407
Peppers, sweet, red, frozen, chopped, unprepared	380
Tamales, masa and pork filling (Hopi)	342
Soup, cream of vegetable, dry, powder	334

Table 3 – Top Food Sources of Lutein & Zeaxanthin, taken from USDA Food Composition Database[35]. Cruciferous vegetables are in bold.

Description	µg/100 g
Kale, frozen, cooked, boiled, drained, without salt	19,697
Kale, frozen, cooked, boiled, drained, with salt	19,697
Spices, paprika	18,944
Kale, cooked, boiled, drained, without salt	18,246
Kale, cooked, boiled, drained, with salt	18,246
Spinach, frozen, chopped or leaf, cooked, boiled, drained, without salt	15,690
Spinach, frozen, chopped or leaf, cooked, boiled, drained, with salt	15,690
Sweet potato leaves, raw	14,720
Dandelion greens, raw	13,610
Spices, pepper, red or cayenne	13,157
Turnip greens, raw	12,825
Spinach, frozen, chopped or leaf, unprepared	12,651
Cress, garden, raw	12,500
Spinach, raw	12,198
Turnip greens, frozen, cooked, boiled, drained, without salt	11,915
Turnip greens, frozen, cooked, boiled, drained, with salt	11,915
Sweet potato leaves, cooked, steamed, without salt	11,449
Sweet potato leaves, cooked, steamed, with salt	11,449
Spinach, cooked, boiled, drained, without salt	11,308
Spinach, cooked, boiled, drained, with salt	11,308
Chard, Swiss, cooked, boiled, drained, without salt	11,015
Chard, Swiss, cooked, boiled, drained, with salt	11,015
Chard, Swiss, raw	11,000
Collards, frozen, chopped, cooked, boiled, drained, without salt	10,898
Collards, frozen, chopped, cooked, boiled, drained, with salt	10,898

2.8. Blood Carotenoid Levels

Although these carotenoids have exhibited anti-cancer effects, their influence on prostate cancer incidence has not been examined to the same degree as lycopene or β-carotene. A meta-analysis of blood carotenoids and prostate cancer risk was carried out in 2007[53]. α-carotene, β-cryptoxanthin, and lutein all had slightly reduced pooled relative risks – 0.97 (95% CI = 0.81-1.16), 0.96 (95% CI = 0.80-1.14) and 0.94 (95% CI = 0.79-1.13), respectively. An increased relative risk was found for blood zeaxanthin 1.20 (0.92, 1.56), but like the other results it failed to reach statistical significance.

2.9. Objective

These four carotenoids have not been the main focus of any investigations of prostate cancer epidemiology, and to our knowledge this is the first time that dietary intake of carotenoids has been examined in a meta-analysis. The aim of this project was to complete a meta-analysis of dietary intake of four carotenoids – α-carotene, β-cryptoxanthin, lutein, and lutein & zeaxanthin – to determine their role in prostate cancer incidence.

3. Materials and Methods

3.1. Search Strategy/Identification of Literature

Between January 13th and February 6th an initial literature review of studies of nutritional factors and prostate cancer (Appendix 2) was performed. Subsequently, it was decided that the focus of this degree project should be dietary intake of carotenoids and risk of prostate cancer. All searches were completed by a single investigator (EL), with consultation from supervisors (KB, JP, AS).

A comprehensive, systematic literature search for relevant studies was completed using electronic databases. The primary database used was PubMed (MEDLINE), and the search comprised all studies published up to February 2014. The Medical Subject Heading (MeSH) terms used in the search were "Prostatic Neoplasms" AND "Carotenoids". Studies were briefly evaluated based on their titles and abstracts. In addition further studies were identified by reviewing the references cited in relevant articles. The results of the search are summarised in the PRISMA[54] Flow Diagram in Figure 5.

Where only abstracts were available the KI Library tool "reSEARCH" was used to locate full texts, and to locate texts found via grey referencing. The "reSEARCH" tool amalgamates content from a number of different sources, including PubMed, CINAHL, Cochrane Library, EMBASE, Google Scholar, MEDLINE, OVID, and Web of Science.

3.2. Inclusion & Exclusion Criteria

Studies whose abstracts were deemed sufficient were considered for further review. Included studies needed to meet the following criteria:

I. Contain a measure of dietary intake of carotenoids (α-carotene, β-cryptoxanthin, lutein, or lutein & zeaxanthin)
II. Case-control, nested case-control (NCC), cohort, or case-cohort studies on human populations
III. Published as an original article
IV. Published in English and with the full text available
V. Contain an appropriate point estimate – odds ratio (OR), relative risk, rate ratio, hazard ratio (HR) – and report 95% CIs

Studies not meeting these criteria were excluded from the analysis. Review articles and dietary intervention trials (RCTSs)[†] were not considered for analysis. In studies with overlapping populations only the most recent study with the largest sample size was considered. Details of the case-control and cohort studies included in the analysis are provided in Tables 4 and 5, respectively.

[†] RCT = randomised controlled trial

3.3. Data Extraction & Evaluation

A total of sixteen studies met the criteria for analysis. Data from these studies was extracted and compiled into tables designed by the investigator. Where a study provided separate adjusted point estimates for different carotenoids – α-carotene, β-cryptoxanthin, lutein, or lutein & zeaxanthin – they were treated as independent studies.

The data that were extracted comprised the name of the first author, year of publication, location of study, study design, sample size, age range of participants, adjusted point estimates for highest versus lowest dietary intakes of carotenoids and corresponding 95% CIs, and adjusted covariates. Further details of dietary assessment methods and exposure assessments were also noted (Appendices 3 and 4, respectively). The extracted data were used to manually examine study quality and also to assess the heterogeneity of studies. These data were reviewed and approved by supervisors.

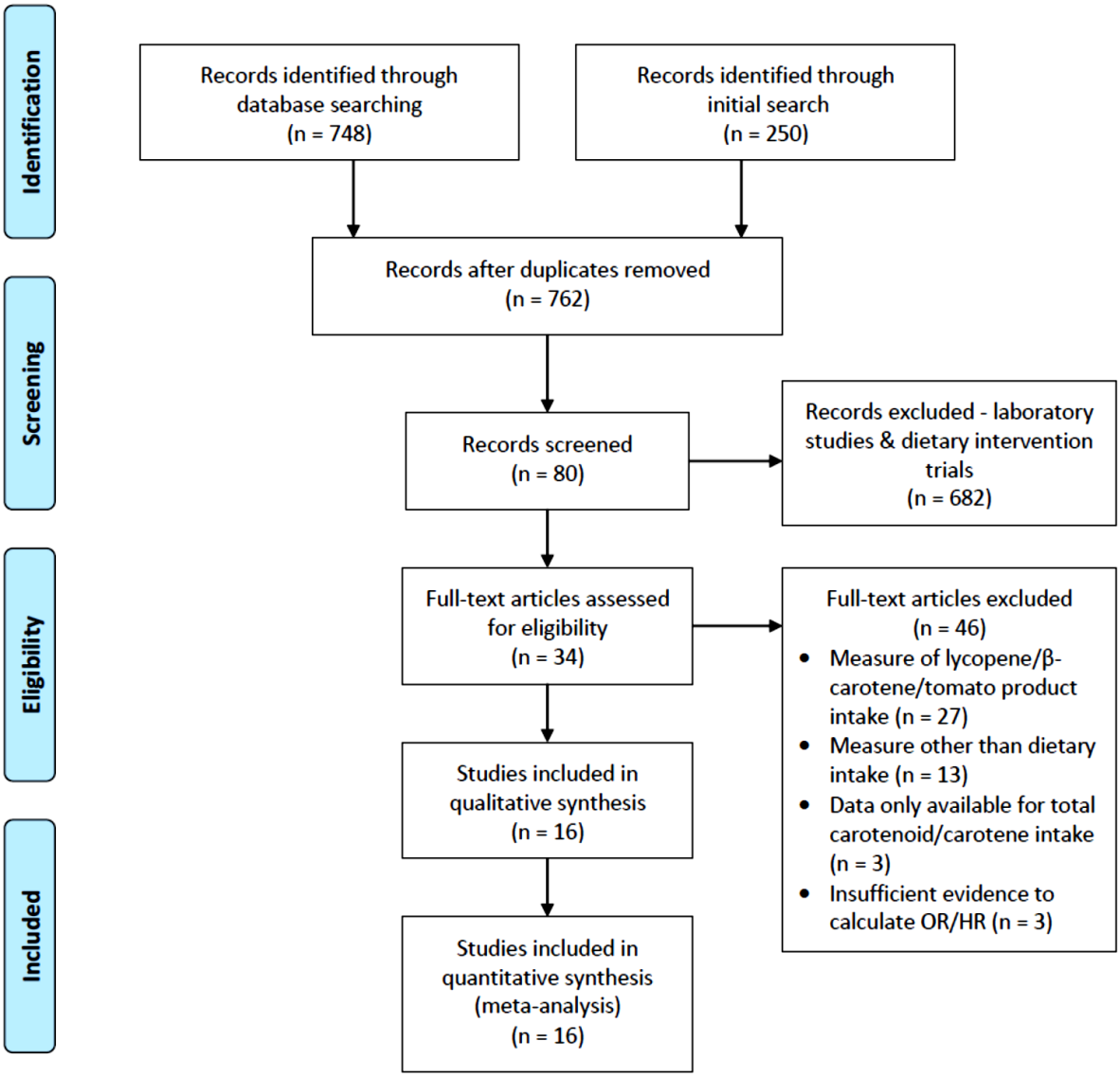

Figure 5 – PRISMA[54] Flow diagram demonstrating the process of study selection

3.4. Statistical Analysis

For statistical purposes prostate cancer is considered a rare disease, thus an OR can be assumed to be approximately the same as a relative risk/risk ratio. However, this does not equate or approximate the rate ratio or HR, as summarised in the equation below:

Odds ratio ≈ (relative risk = risk ratio) ≠ (rate ratio = hazard ratio)

Studies reporting the use of Cox regression to calculate point estimate were taken as a report of HR. These studies were analysed separately to studies using OR as their point estimates to avoid confounding of results. Adjusted point estimates (OR or HR) and corresponding 95% CIs were pooled for highest versus lowest dietary intakes of α-carotene, β-cryptoxanthin, lutein, or lutein & zeaxanthin, respectively.

A custom Do-File was created (Appendix 5) for statistical analysis of each carotenoid for pooled OR and HR respectively. Publication bias was also assessed mathematically and graphically. Log ORs/HRs and the log of the upper limit of the 95% CI for each study in the relative stratified analyses were generated to calculate standard errors. The meta-analysis reported results in log form, but these were converted to the natural form as shown in Appendix 6. Statistical significance was considered when $p<0.05$ for pooled point estimates and when I^2 >50% for heterogeneity. Publication bias was considered present when $p<0.05$. All statistical analyses were carried out with Stata Statistical Software, version 13.1 (StatCorp LP, College Station, TX, USA).

4. Results

4.1. Study Characteristics

A total of sixteen studies satisfied the criteria for inclusion in this meta-analysis (Tables 4 and 5). Many of the studies overlapped in their measure of different carotenoids; fifteen provided a measure of α-carotene intake, thirteen for β-cryptoxanthin, seven for lutein, and eight for lutein & zeaxanthin. All studies included in this analysis compared ORs or HRs of prostate cancer incidence in the highest compared to the lowest intakes of the respective carotenoids. However these are merely qualitative descriptors, as quantification of exposure assessments varied greatly between included studies (see Appendix 4). Publication dates of the included studies ranged from December 1995 to February 2014.

Study design also varied widely among included studies: there were nine case-control (five population-based and four hospital-based), four cohort, two case-cohort, and one nested case-control study (NCC). The NCC and one of the cohort studies were conducted within the same study population, but were both included due to differences in their designs and sub-cohort selection. All studies adjusted for age, and he most common adjustments among included studies were for total energy intake, body mass index (BMI), family history of prostate cancer, education, race/ethnicity, location (within respective study population), smoking, and physical activity. The majority of studies were completed in Western countries; ten from North America, two from Europe and one from Australia. Two were from Asian populations and one was from South America.

4.2. Dietary Assessment

A summary of dietary assessment methods is shown in Appendix 3. All but one study[61] used food frequency questionnaires (FFQs) to assess diets of participants. In eight of the sixteen studies (including all 6 cohort/case-cohort studies) these FFQs were completed via interview and the remaining eight were self-administered. Nine of the questionnaires had been previously validated, and one had been developed specifically for use in epidemiological studies of the local population. The number of food/beverage items in these FFQs ranged from 35 to 180, and were divided into various groupings based on each study's individual design.

Different methods were used by each study to measure portion size and asses consumption frequency of foods. The reference timeframe for the usual dietary intake was set at a minimum of 12 months prior to the date of assessment. United States Department of Agriculture (USDA)[35] sources were most commonly used to calculate nutrient intake estimates, with some studies using native food composition databases. Six studies also assessed vitamin and mineral supplementation use among participants.

Table 4 – Characteristics of Case-Control Studies of Dietary Intake of Carotenoids Included in Meta-analysis

Reference (Year)	Design	n Cases	n Controls	Location	Age Range (Mean)	Dietary Assessment Method	PCa Definition	Carotenoids Investigated
Rohrmann (2007)[48]	NCC	6092	18373 (HPFS)	USA	40-75	Validated, semi-quantitative FFQ, 131 items, self-administered	BPH – several different criteria	α-C, β-Cr, L&Z
McCann (2005)[55]	PCC	433	538	New York	not reported	FFQ, 172 items, interview	Primary histologically confirmed prostate cancer	α-C, β-Cr, L
Bosetti (2004)[56]	HCC	1294	1451	Italy	46–74	Validated FFQ, 78 food beverages & recipes, interview	Histologically confirmed carcinoma of the prostate	α-C, β-Cr, L&Z
Hodge (2004)[57]	PCC	858	905	Melbourne, Sydney & Perth, Australia	<70	FFQ, 121 items, interview	Histologically confirmed prostate cancer, Gleason score ≥5	α-C, β-Cr, L&Z
Jian (2004)[58]	HCC	130	274	Hangzhou, SE China	(cases 72.7, controls 71.4)	Validated, adapted FFQ, 130 items, interview	Histologically confirmed adenocarcinoma of the prostate	α-C, β-Cr, L&Z
Lu (2001)[51]	HCC	65	132	New York	(cases 59.98, controls 41.9)	NCI HHHQ short dietary questionnaire, interview	Pathologically confirmed diagnosis of prostate adenocarcinoma	α-C, β-Cr, L
Cohen (2000)[59]	PCC	152	145	King County, WA, USA	40-64	FFQ, 98 items, self-administered	Histologically confirmed prostate cancer	α-C, β-Cr, L&Z
Deneo-Pellegrini (1999)[60]	HCC	175	233	Uruguay	40-89	FFQ, 64 items, interview	Histologically verified prostatic adenocarcinomas	α-C, L
Jain (1999)[61]	PCC	617	636	Ontario, Quebec, & British Colombia, Canada	(69.8 cases, 69.9 controls)	validated quantitative diet history, interview	Recent, histologically confirmed diagnosis of adenocarcinoma of the prostate	α-C, β-Cr, L
Meyer (1997)[62]	PCC	215	593	Quebec City, Canada	≥45	Validated FFQ, 143 items, interview	Preclinical prostate cancer – histologically or screen detected	α-C, L

NCC = nested case-control, PCC = population case-control, HCC = hospital case control, HPFS = Health Professionals Follow-Up Study, FFQ = food frequency questionnaire, NCI = National Cancer Institute (http://www.cancer.gov/), HHHQ = Health Habits and History Questionnaire, α-C = α-carotene, β-Cr = β-cryptoxanthin, L = lutein, L&Z = lutein & zeaxanthin

Table 5 – Characteristics of Cohort/Case-Cohort Studies of Dietary Intake of Carotenoids Included in Meta-Analysis

Reference (Year)	n Cases	n Cohort	Cohort Name	Location	Age Range (Mean)[a]	Mean Follow-Up (Years)	Dietary Assessment Method	PCa Definition	Carotenoids Investigated
Umesawa (2014)[63]	143	15,471	JACC	Japan	40-79	(16 – median)	Validated FFQ, 35 items, self-administered	Incident PCa	α-C
Agalliu[b] (2011)[64]	661	1,864 sub cohort	CSDLH	Canada	(70 – age at diagnosis)	4.3 (cases), 7.7 (controls)	Validated, adapted FFQ, 166 items, self-administered	Incident prostate cancer	β-Cr, L&Z
Kirsh (2006)[65]	1,338	29,361	PLCO	USA	55–74	4.2 (max 8)	FFQ, 137 items, self-administered	Prostate cancer diagnosis	α-C, β-Cr, L&Z
Stram (2006)[66]	3,922	78,564	MEC	USA	45-75	7	FFQ, 180 items, self-administered	Incident prostate cancer	α-C, β-Cr, L
Schuurman[b] (2002)[67]	642	1,525	NLCS	Holland	55-69	6.3	Validated, semi-quantitative FFQ, 150 items, self-administered	Incident prostate carcinoma	α-C, β-Cr, L&Z
Giovannucci (1995)[68]	812	47,894	HPFS	USA	40-75	6	Validated FFQ, 131 items, self-administered	Adenocarcinoma of the prostate	α-C, β-Cr, L

[a] = at baseline of enrolment into cohort, [b] = case-cohort study design, JACC = Japan Collaborative Cohort Study, CSDLH = Canadian Study of Diet, Lifestyle and Health, PLCO = Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, MEC = Multi-ethnic Cohort Study, NLCS = Netherlands Cohort Study, HPFS = Health Professionals Follow-Up Study, FFQ = food frequency questionnaire, α-C = α-carotene, β-Cr = β-cryptoxanthin, L = lutein, L&Z = lutein & zeaxanthin

4.3. Dietary Carotenoid Intake and Prostate Cancer Risk

Results of the meta-analyses of four dietary carotenoids – α-carotene, β-cryptoxanthin, lutein, and lutein & zeaxanthin – are summarised in Table 6. Results of statistically significant analyses are displayed as Forrest plots in Figures 6 and 8-10. Full details of analysis of each of the four carotenoids, including Forrest and Funnel Plots can be found in Appendix 6.

Table 6 – Summary of Results of Meta-analysis of Four Carotenoids and Risk of Prostate Cancer

Carotenoid	Point Estimate	# Studies	Pooled OR/HR	P value	I^2 (%)	P Begg's	P Egger's
α-Carotene	OR	10	**0.92 (0.84-1.00)**	**0.04**	6.3	0.15	0.06
	HR	5	0.94 (0.87-1.02)	0.15	0.0	0.22	0.62
β-Cryptoxanthin	OR	8	**0.91 (0.83-0.99)**	**0.03**	**76.9**	0.71	0.67
	HR	5	0.98 (0.91-1.06)	0.65	40.3	0.31	0.29
Lutein	OR	5	**0.76 (0.60-0.97)**	**0.03**	0.0	0.46	0.28
	HR	2	1.00 (0.91-1.10)	0.97	0.0	1.00	-
Lutein & Zeaxanthin	OR	5	**0.82 (0.75-0.89)**	**0.00**	**81.9**	0.22	0.29
	HR	3	0.95 (0.82-1.09)	0.96	0.0	1.00	0.80

Statistically significant results in bold

4.4. α-Carotene

Fifteen studies of dietary intake of α-carotene were included in this analysis; ten case-control/NCC, and five cohort/case-cohort. A reduced risk of prostate cancer incidence was identified for α-carotene in both study categories. However, only the results for the case-control/NCC analysis reached statistical significance. Analysis showed a pooled OR of 0.92 (95% CI = 0.84-1.00, p = 0.04) for higher α-carotene intakes compared to lower (Figure 6). No significant heterogeneity was detected between studies (6.3%), and tests for publication bias reached only borderline significance for case-control/nested case-control studies (p value for Begg's test = 0.15; p value for Egger's test 0.06; Figure 7).

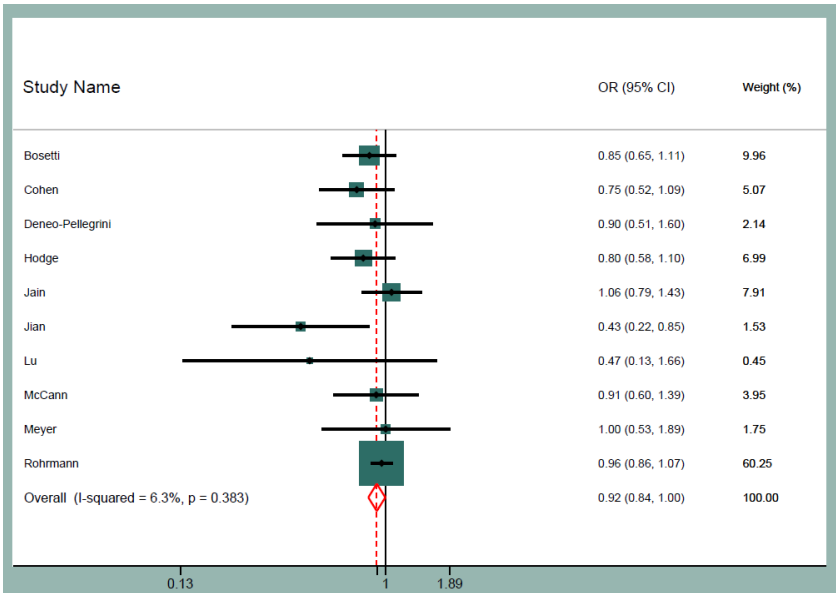

Figure 6 - Forrest Plot for Meta-analysis of Case-control/NCC Studies of α-Carotene and Risk of Prostate Cancer

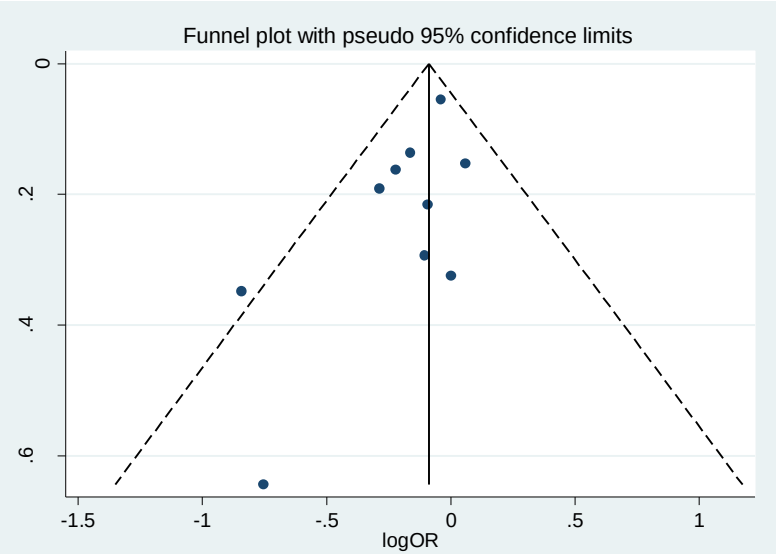

Figure 7- Funnel Plot for Test of Publication Bias in Case-control/NCC Studies of α-Carotene and Prostate Cancer

4.5. β-Cryptoxanthin

Thirteen studies included a measure of dietary intake of β-cryptoxanthin. A reduced risk of prostate cancer was recorded in both categories of study design. However, as with α-carotene, the analysis of five cohort/case-cohort studies did not reach statistical significance (pooled HR = 0.98; 95% CI = 0.91-1.06). A statistically significant reduced risk of prostate cancer was recorded in the analysis of the eight case-control/NCC studies (pooled OR = 0.91; 95% CI = 0.83-0.99; p=0.03; Figure 8). There was significant heterogeneity between studies in this analysis (I^2 = 76.9%, p = 0.000), and possible reasons for this are discussed in section 5.4. Publication bias was not detected in neither case-control/NCC (p Begg's =0.71; p Egger's = 0.67) nor cohort/case-cohort studies (p Begg's =0.31; p Egger's = 0.29).

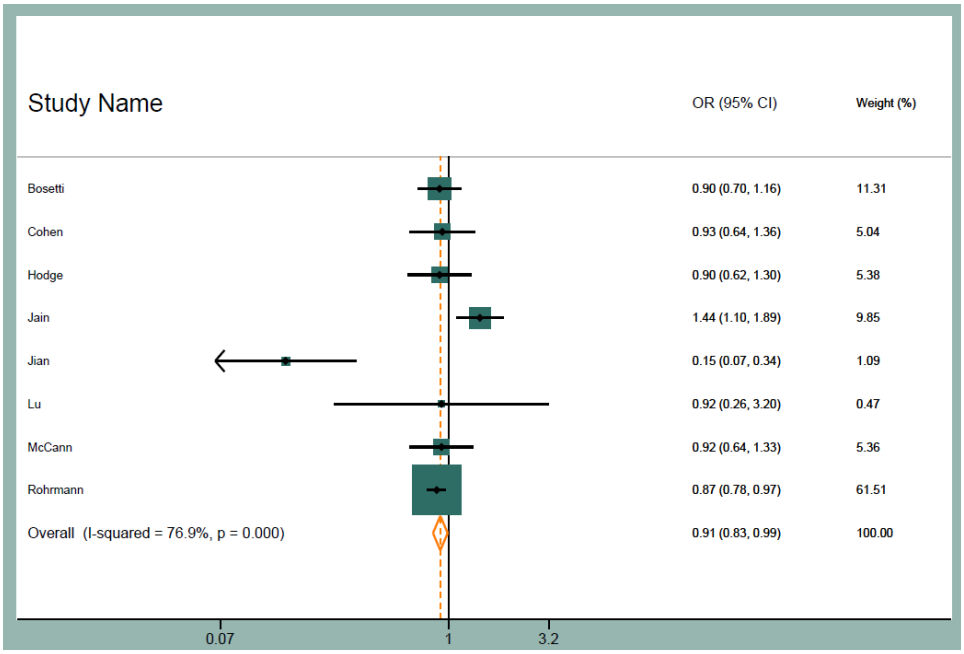

Figure 8 - Forrest Plot for Meta-analysis of case-control/NCC Studies of β-Cryptoxanthin and Prostate Cancer

4.6. Lutein

Only seven studies included a measure of dietary intake of lutein – five case-control and two cohort studies. In the cohort study analysis, no association was found for the highest category of lutein intake (pooled HR = 1.00; 95% CI =0.91-1.10; p = 0.97), but this result is limited due to the small number of studies included. There was a statistically significantly reduced risk of prostate cancer found in the analysis of case-control studies (pooled OR = 0.76; 95% CI = 0.60-0.97; p = 0.03; Figure 9). No heterogeneity was detected in either the case-control (0.0%) or cohort studies (0.0%), and no publication bias was detected among case-control studies in either of Begg's (p = 0.46) or Egger's tests (p = 0.28). Assessment of publication bias in cohort studies of lutein was not possible due to the limited number of studies available for analysis.

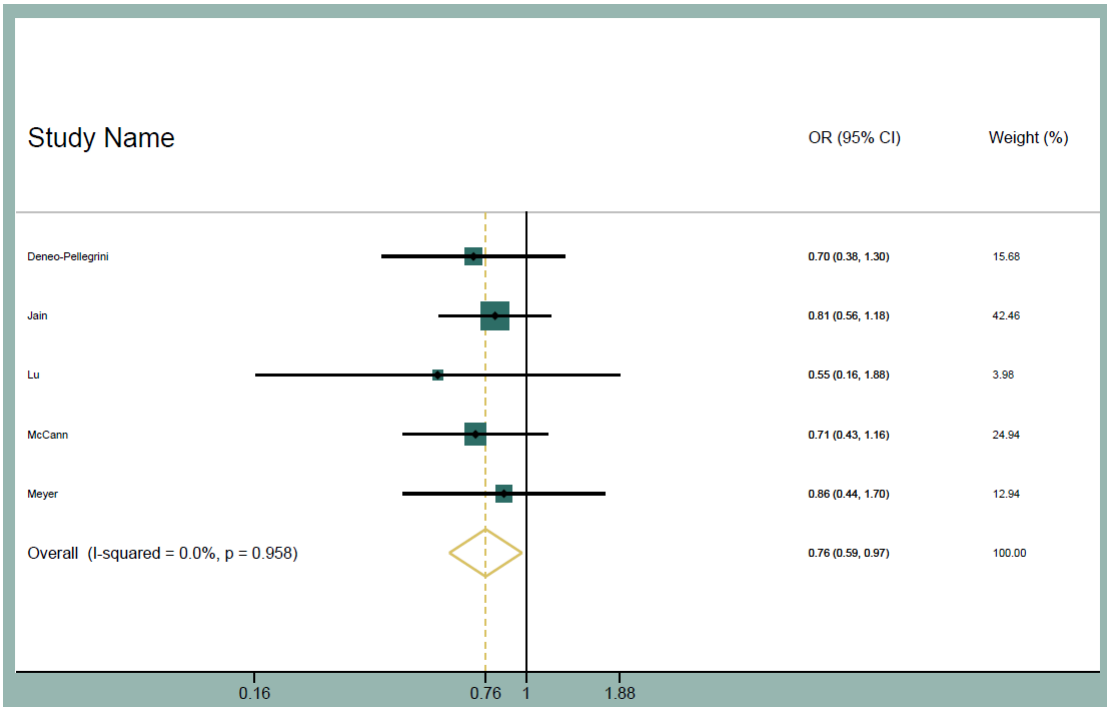

Figure 9 - Forrest Plot for Meta-analysis of Case-control/NCC Studies of Lutein and Risk of Prostate Cancer

4.7. Lutein & Zeaxanthin

Eight studies examined the intake of lutein and zeaxanthin combined – five case-control/NCC and three cohort/case-cohort. Similar to the other cohort/case-cohort analyses the pooled HR did not reach statistical significance (0.95; 95% CI = 0.82-1.09; p = 0.96), and no heterogeneity was observed (0.0%). In the case-control/NCC analysis, a statistically significant reduced risk of prostate cancer was associated with the highest intakes of lutein & zeaxanthin (pooled OR 0.82; 95% CI = 0.75-0.89; p = 0.000; Figure 10). However, there was significant heterogeneity (81.9%, p = 0.000), which is discussed along with β-cryptoxanthin in section 5.4. No significant publication bias was detected among case-control/NCC (p

value for Begg's test = 0.22; p value for Egger's test 0.29), or cohort/case-cohort studies. Results of the publication bias analyses among cohort/case-cohort studies is limited due to the low number of studies available for analysis (p value for Begg's test = 1.00; p value for Egger's test 0.80).

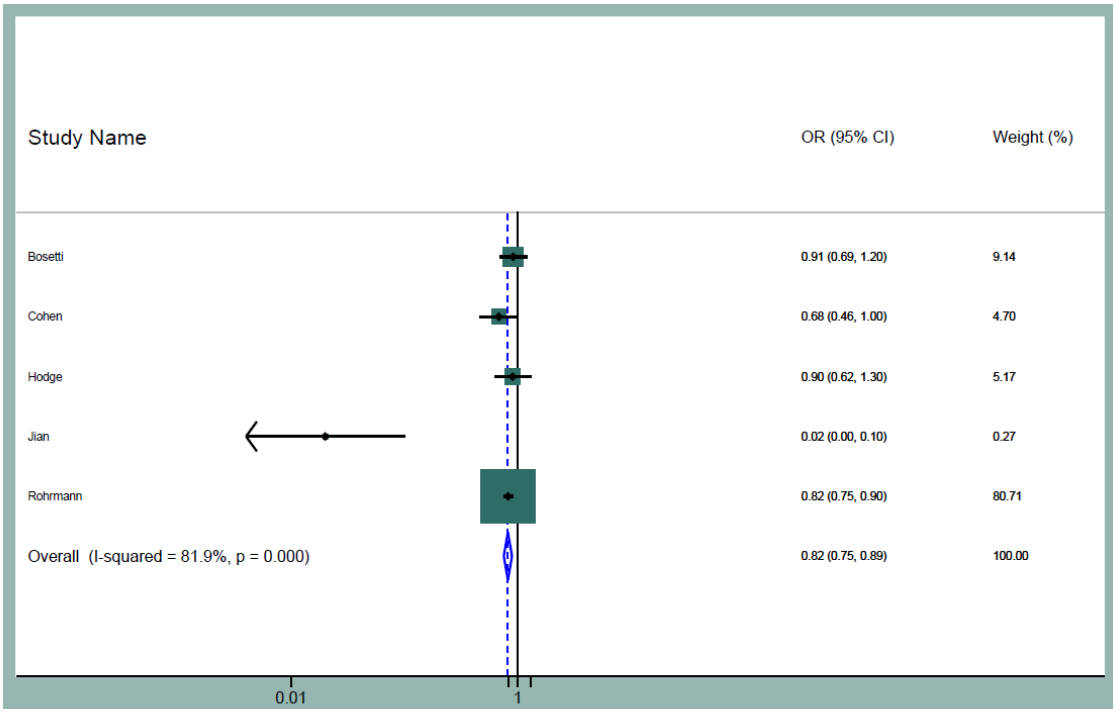

Figure 10 - Forrest plot for Meta-analysis of case-control/nested case-control studies of lutein & zeaxanthin and Risk of Prostate Cancer

Overall these results demonstrate an inverse association of increasing intakes of α-carotene, β-cryptoxanthin, lutein, and lutein & zeaxanthin.

5. Discussion

To our knowledge, this is the first time that these four carotenoids – α-carotene, β-cryptoxanthin, lutein, and zeaxanthin – have been the focus of a meta-analysis of dietary intakes and prostate cancer incidence, despite there being ample data available from multiple epidemiological investigations. The majority of studies took place in Western populations, which may reflect their higher incidences of prostate cancer (Figure 2). A reduced risk of prostate cancer was found for higher intakes of all four carotenoids, however only the results for case-control/NCC studies reached statistical significance (see sections 4.3-4.7). Lutein showed the strongest protective effect, with a reduced incidence of 24% found among those with higher intakes in case-control studies. Combined intakes of lutein & zeaxanthin also reduced incidence at higher intakes, as did α-carotene and β-cryptoxanthin. However the results of these analyses are disputed in section 5.5 below.

5.1. Measures of Association

Studies were categorized based on their measure of association, OR or HR. Studies reporting a measure of risk ratio or relative risk were reviewed to see if there methods were statistically sound. Three studies used "relative risk" as their outcome measure but were reassigned HRs due to their statistical reasoning. Two of these[65, 66] were because they used Cox regression and, another[68] for using rates in their calculation and proportional hazards regression. Schuurman et al[67] used "rate ratio" for their outcome measure and assumed exponentially distributed survival times, which was judged to be a HR calculation. Two studies reported an outright HR measurement and were accepted as such[63, 64]. All studies reporting HR were either cohort or case-cohort, whereas all studies using OR as their outcome measure were case-control or NCC. Due to these differences in study design and point estimate measures, is was not possible to measure the cumulative effect among all studies for each carotenoid.

5.2. Study Design

Rohrmann et al[48] carried out a nested case-control study in their investigation, using data from the Health Professionals Follow-Up Study. An NCC study is one where subjects are sampled from an already assembled epidemiological cohort study, in which the sampling depends on disease status[69]. Case-cohort designs are similar to nested case-control studies, except that the controls are randomly selected from the full cohort without matching. Case-cohort studies do however allow for the evaluation of multiple disease endpoints[70], which was ideal for Agalliu et al[64], as they examined individual pro- and anti-oxidants as well as their cumulative influence on prostate cancer risk. Similarly Schuurman et al[67] assessed the intakes of certain nutrients and incidence of prostate cancer among drinkers and non-

drinkers. Characteristics of case-control/NCC and cohort/case-cohort studies are shown in Tables 4 and 5, respectively.

Significantly reduced incidences of prostate cancer were found for each of α-carotene, β-cryptoxanthin, lutein, and lutein & zeaxanthin in all case-control/nested case-control studies. Though the results for cohort/case-cohort studies show reduced risks (except for lutein, which showed no association; pooled HR = 1.00; 95% CI = 0.91-1.10), they did not reach statistical significance. This pattern has been seen in previous meta-analyses of dietary intakes and prostate cancer risk[19, 71]. A possible reason for this could be the high number of hospital-based case-control studies included in this analysis, as these are more vulnerable to selection and Berkson's bias[72] than population case-control studies and cohort studies.

Case-control studies are generally considered less consistent, and rank lower on the hierarchy of evidence[73]. This could be because case-control studies are vulnerable to oversampling, in that the number of cases (and matched controls) may not be representative of disease rates in the entire population. Location of studies may be another reason for this, as studies carried out in low-risk populations (e.g. Asia) may not contain a sufficient number of cases to be representative of total incidence worldwide. The largest cohort study by Stram et al[66] came from the Multi-ethnic Cohort Study[74], and contained 3,922 cases and 78,564 controls. A study like this would be more representative of prostate cancer incidence in the total population than a case-control study. However, the purpose of this analysis was to determine the influence of dietary carotenoids on prostate cancer risk, and case-control studies are an invaluable source of information in epidemiological investigations such as this one.

5.3. Dietary Assessment

Despite the high quality of dietary assessment methods among studies in this analysis, there was a lot of inconsistency (see Appendix 3). All but one study[61] used food frequency questionnaires (FFQs) to assess participants' diets, and although these FFQs were tailored to the populations being scrutinized, there is little opportunity to make comparisons between studies. Umesawa et al[63] only included 35 foods in their FFQ, and gave no information about how the nutrient and carotenoid contents were calculated. Although the FFQ used for that study had been previously validated, this does not sufficiently reflect the wide variety of foods containing high levels of different carotenoids. Half of the studies included measured between 121 and 166 food items in their FFQs. In an attempt to reduce recall bias[75], most studies asked participants to estimate their consumption over the past year. This would also account for any changes in diet that may have occurred following prostate cancer diagnosis[76].

Ten studies utilized the UDSA Nutrient Database to calculate the carotenoid contents of the food they investigated. Two studies used native food composition databases (Italian[56] and Dutch[67]), and

another[64] adapted the UDSA data to reflect local food availability and fortification laws. Adaptation of nutrient estimates or use of local estimates is favourable, as soil quality and nutrient content vary widely throughout the world, and the use of databases from other countries may result in incorrect calculations of nutrient composition of foods. Two studies used composition data from Mangels et al[77], and a further two utilised the Nutrition Data System developed by the University of Minnesota[78]. Six studies also examined supplement use, which could have contributed to higher carotenoid intakes. Supplementation is further discussed in section 5.9.

There were also significant differences in the techniques used to calculate consumption frequency and portion size. This may in some part explain the disparities in the categories of intake used for analysis (see Appendix 6). Point estimates (OR or HR) were taken for the highest versus lowest level of intake, be they quintiles, quartiles, mean, or median values. Stram et al[66] defined quintile boundaries based on micrograms per 1,000 kilocalories, preventing comparison to other studies which estimated daily intakes, and Meyer et al[62] did not report the quartile boundaries used in their study. Using lutein as an example, the quartile boundaries set by McCann et al[55] are particularly high. Their first/lowest quartile of intake (≤3029µg/d) was higher than the fourth /highest quartile used by Jain et al[61] (>2684 µg/d). These exceptionally high intake measures could account for the significant trend found for increased lutein intake reported my McCann et al ($p = 0.01$). In all cases, disparities in categorizing intake levels contributed to heterogeneity among studies.

5.4. Heterogeneity

Further heterogeneity between studies can be attributed to the adjustments used in point estimate calculations (see Appendix 4). All sixteen studies adjusted for age, an already established risk factor for cancer[79]. The next most common adjustments were for BMI (11/16), energy intake, family history of prostate cancer (10/16), education, location (6/16), smoking, race/ethnicity (5/16), fat intake, alcohol intake (4/16), physical activity, socioeconomic status (3/16), and marital status (2/16). The number of adjustments applied in each study also varied, with most studies adjusting for four to fourteen covariates. Giovannucci et al[68] only adjusted for age and energy intake, whereas Jain et al[61] adjusted for a total of 23 covariates, many of them log-converted amounts for other dietary factors.

The sample sizes also varied between the studies included in the analysis. Among cohort studies, the largest cohort study (Stram et al[66], The Multi-ethnic Cohort Study[74]) had over five times more participants than the smallest one (Umesawa et al[63], Japan Collaborative Cohort[80]). However, there were only 143 cases of prostate cancer in the JACC study, compared to 3,922 in the MEC one. This rate is over 24 times higher, though probably attributable to the lower incidence rates in Japan[1]. In case-control

studies, the smallest one had just 65 cases and 132 controls[51], while the largest one had 1294 cases and 1451 controls[56].

Rates for prostate cancer are low among men below the age of 45 (9.2 per 100,000 for men aged 40-44 years), but increase to 984.8 per 100,000 in men aged 70-74 years[79]. The majority of studies enrolled men aged between 45 and 75 and only one study included men below the age of forty[51]. Only one study allowed men over the age of eighty[60], though McCann et al[55] did not report the ages of participants.

5.5. Exclusion of Jian et al[58]

Although there was a reduced risk of prostate cancer found in the case-control/NCC analyses of β-cryptoxanthin (pooled OR = 0.91) and lutein & zeaxanthin (pooled OR 0.82), there was substantial heterogeneity observed in these analyses. The results of the I^2 tests showed 76.9% and 81.9% heterogeneity among studies for β-cryptoxanthin and lutein & zeaxanthin, respectively. Possible sources for this heterogeneity have been discussed above, but manual examination of the ORs for the individual studies involved in these analyses led us believe that the study by Jian et al[58] could be a potential outlier.

To test this hypothesis we repeated the meta-analysis of case-control/NCC studies for β-cryptoxanthin and lutein & zeaxanthin excluding the results from Jian et al (Appendix 7). For β-cryptoxanthin, heterogeneity was reduced to 47.7%, though the reduced risk of prostate cancer did lose statistical significance (pooled OR 0.93; 95% CI = 0.85-1.01; p = 0.08). In the case of lutein & zeaxanthin, exclusion of the study by Jian et al completely eliminated heterogeneity (0.0%). The protective effect of higher intakes was slightly diminished, but maintained statistical significance (pooled OR 0.83; 95% CI = 0.76-0.90; p = 0.000). There was no detectible publication bias found for neither β-cryptoxanthin nor lutein & zeaxanthin, in repeat Begg's or Egger's tests (results not shown).

Though no significant heterogeneity was found among case-control/nested case-control studies of α-carotene (6.3%), we repeated the analysis excluding Jian et al. Heterogeneity was reduced to 0.0%, but like β-cryptoxanthin, the results lost statistical significance (pooled OR 0.93; 95% CI =0.85-1.01; p = 0.08). We can conclude that the results reported by Jian et al are strongly influential to the analysis of α-carotene and β-cryptoxanthin. Results for lutein & zeaxanthin however remained robust, and their influence in prostate cancer should be further examined in future studies.

Upon initial manual examination of Jian et al, no significant differences between other studies were detected. Possible reasons for the exceptionally low ORs found in this study include the large reference recall period for dietary assessment interview (5 years before diagnosis/interview), or the fact that 65.3% of control were recruited from the inpatient urology department of the hospitals involved. This is the only case-control study carried out in Asia, the region with the lowest rates in the world, and

this is reflected in the relatively small sample size. Although the FFQ used in this study was adapted from questionnaires used in four previous studies, it had been previously validated in native Chinese populations. The authors assume that participants in the study were representative of the Zhejiang population, and the FFQ used contained traditional Chinese units of measurement ("Liang"). It is possible that the low rates of prostate cancer in the region examined by Jian et al contributed to the exceptionally low ORs found for higher carotenoid intakes.

5.6. Prostate Cancer Definition

There was some variance in the definition of prostate cancer between studies. Though not all studies specify, we can assume that "prostate cancer" refers to adenocarcinoma of the prostate. In many of the studies, the prostate cancer needed to be histologically or pathologically confirmed before a participant could be considered a case. In the cohort studies, subjects with prostate cancer at enrolment were excluded from their investigations. Incident prostate cancer was used to detect cases in these studies, usually through linkages with local cancer registries.

We included two studies that used benign prostatic hyperplasia (BPH) as their inclusion criterion. Over 90% of men aged 85 show histological evidence of BPH, and approximately one in four men will require medical care for the condition by age 80[81]. Though the link between BPH and prostate cancer has been disputed[82], both conditions have high prevalence worldwide and share many pathophysiological properties. Finasteride is a 5α-reductase inhibitor used to treat BPH[83], and this drug was also shown to reduce overall risk of prostate cancer by 30% in the Prostate Cancer Prevention Trial[84]. Lycopene has been shown to reduce BPH progression[85], and so the effects of other carotenoids on the condition warrants further investigation.

Two of the studies used BPH as their inclusion criterion. Meyer et al[62] assessed two groups; one was men hospitalised for transurethral prostatectomy (TURP) where prostate cancer was discovered in resected tissue, and the other was men who took part in a screening program and were referred for radical treatment during the study period. These two groups were combined for the analysis of nutrient intake, and cases are referred to as "preclinical prostate cancer" throughout the paper. Rohrmann et al[48] carried out an NCC study within the Health Professionals Follow-Up Study (HPFS), which involved a number of different follow-up assessments during the study period. They examined BPH, defined in two different ways. Diagnosis of "total BPH" was based on a history of surgery for an enlarged prostate, high-moderate to severe lower urinary tract symptoms (AUASI[‡] score ≥15) and use of medications (α-blockers, finasteride) to treat BPH. Diagnosis of "incident BPH" was based reports of surgery/symptoms in follow-ups after 1994. In this analysis we took the OR for "total BPH".

[‡] AUASI = American Urological Association Symptom Index

5.7. Publication Bias

We assessed whether or not publication bias was present using Begg's and Egger's tests and by generating funnel plots (see Appendix 5). Publication bias is the selective publication of studies based on favourable characteristics[86], for example studies reaching statistical significance, popularity of the topic, having a sponsor, and studies published in English. The Begg's test assesses the presence of association between the effect estimates and their variances[87], with significant correlation indicating publication bias is present. This test however is unreliable when the number of studies is small, so we also used Egger's test[88] which is more specific. Egger's test plots a regression line between precision of the studies and the standardized effect, and measures correlation mathematically to generate a p value like Begg's test.

No publication bias was detected in any of the stratified analyses. Tests for α-carotene reached borderline significance in the Egger's test (0.06) in case-control/nested case-control studies, and slight asymmetry (caused by two outliers) was noted in the funnel plot (Figure 7). The probability of publication bias being influential in this analysis is low, as none of the carotenoids examined here have been the main topic of any epidemiological investigations into prostate cancer. However, because this project specified non-English papers as an exclusion criterion, publication bias has been introduced. This was due to time constraints, and can be amended for future studies.

5.8. Limitations of Current Analysis

Nutritional epidemiology investigations are always quite limited in their power due to the retrospective nature of dietary recall, and the limited timeframe to complete this project means that there are further limitations in the methodology. Firstly, all reviews, investigation procedures and data extraction were carried out by a single investigator (EL). This introduces the possibility of bias in assessment and recording, and ideally the literature review should have been completed in tandem with another investigator. Secondly, publication bias was introduced as only studies published in English were considered. Also no contact with authors was made when full texts were unavailable, which could potentially have added to our results pool. Thirdly, there was significant heterogeneity among studies, particularly in exposure measurements and adjusted covariates. Excluding Jian et al as an outlier did reduce percentage heterogeneity in all categories affected, but led to the results of case-control/NCC analyses of α-carotene and β-cryptoxanthin losing statistical significance.

5.9. Supplementation

Six of the studies included in this meta-analysis examined supplement use among participants, which could have contributed to carotenoid intakes (see Appendix 3). A recent systematic review and

meta-analysis[89] assessed multivitamin use and prostate cancer occurrence. Neither multivitamin supplementation nor use of individual vitamins or minerals (vitamin E, zinc, selenium, and β-carotene) affected the overall occurrence of prostate cancer. Mortality and incidence of high-grade/metastatic prostate cancer were not affected either, and though there was considerable heterogeneity between the studies, stratified analysis of high-quality studies returned similar results. Another review and meta-analysis of RCTs[90] examined the influence of supplementation with non-herbal dietary supplements and vitamins on prostate cancer patients. They found that no evidence that dietary supplements reduced PSA levels, though two trials using mixtures including carotenoids, lycopenes, and antioxidants (among many others) did significantly reduce PSA levels.

Carotenoid supplementation has not been the subject of many prostate cancer prevention trials. A Cochrane review of lycopene supplementation[91] found that there was insufficient evidence to either support or refute the use of lycopene for the prevention of prostate cancer. Further evidence is required to determine whether carotenoid supplementation is a viable preventative mechanism for prostate cancer incidence or progression.

5.10. Synergy

Despite a lack of evidence from RCTs about the use of carotenoids as a chemoprevention mechanism for prostate cancer, epidemiological investigations of high carotenoid food have returned significant results. As mentioned in section 2.3, lycopene has been extensively investigated as a possible preventative agent for prostate cancer. However, the results of studies investigating foods with high lycopene concentrations, such as tomatoes, have returned even more favourable results. The meta-analysis by Chen et al[32] showed greater reductions in ORs for higher intakes of tomatoes than for lycopene intake. Similarly, the most recent meta-analysis of cruciferous vegetable intake[19] demonstrated a relative risk of 0.90 (95% CI = 0.85-0.96) for higher intakes compared to lower, and many cruciferous vegetables are high in lutein and zeaxanthin (Table 3).

Studies involving whole foods or food groups tend to show greater risk reduction of prostate cancer, and are more often statistically significant than studies of nutrients alone. Carrots[24], tomatoes[32], and cruciferous vegetables[19] have all been shown to reduce prostate cancer incidence in high consumers. As shown in Tables 1-3, these foods contain high levels of carotenoids. It is possible that these foods contain other biomolecules that strengthen the protective effect of carotenoids in a synergistic way. These studies reinforce the findings made here, that higher carotenoid intakes reduce prostate cancer risk.

5.11. Carotenoid Bioavailability

Tables 1-3 show the top dietary sources of α-carotene, β-cryptoxanthin, and lutein & zeaxanthin, respectively. From these tables we can see that there is significant variance between contents based on their preparation and cooking methods. In the case of α-carotene, carrots make up thirteen of the top 25 food sources. Dehydrated carrots, the top dietary source of α-carotene, have over 4 times the amount of α-carotene as raw carrots. Thermal processing of tomato products is an effective way to increase their carotenoid concentrations[92]. Giovannucci et al[68] recorded a relative risks of 0.66 (95% CI = 0.49-0.90) for men who consumed 2-4 servings of tomato sauce per week compared to those who had none (p for trend 0.001). Protective effects can also be greater for different varieties of foods that reduce risk[93].

Different methods of cooking or preparing foods can have a significant impact on the bioavailability of carotenoids in foods. As they are lipid soluble, it has been hypothesised that increasing fatty acid intake as well as carotenoids can improve their health benefits. A 2005 study[94] found that addition of avocado (in fruit or oil form) to salads and salsa enhanced absorption of α-carotene and lutein ($p < 0.01$). Although saturated fat has been shown to increase prostate cancer risk at higher intakes[23], other fatty acid subgroups have been shown to decrease risk (see Appendix 1), and higher quality dietary fats can contribute to better overall health outcomes[95].

5.12. Interpersonal Differences

Epidemiological investigations and meta-analyses give generalised results on how different factors can affect disease outcomes. There are many confounding factors in prostate cancer development, evident by the differences in adjusted covariates between studies (see Appendix 4). Interpersonal differences between members of a population can influence an individual's chance of developing a disease. Different genotypes[96], ethnicities[97], and lifestyle choices (see section 2.1) carry different risks of developing prostate cancer, and thus the influence of carotenoids as a preventative tool will be variable.

Plasma carotenoid concentration and dietary intake are correlated[98], though differences in plasma levels may be due to the different uptake rates among other tissues. One study[99] found that body fat influenced the tissue distribution of carotenoids, with significantly higher concentrations in abdominal adipose tissue compared to the buttock and thigh. Another study[100] found that lycopene and β-carotene were also found in high concentrations in skin compared to lutein and zeaxanthin, and total carotenoids were significantly correlated in skin and plasma. Strong associations between serum and colon measurements of α-carotene and β-cryptoxanthin have also been observed[101]. Furthermore, a number of different carotenoids have been found to be intercorrelated in prostatic tissue[102], with disparities in

concentrations among benign and malignant samples. Although all these measures are correlated, differences in metabolism may account for disparities between subjects.

5.13. Plant-Based Diets

There is substantial evidence to suggest that vegetarian or vegan diets improve overall health[103, 104]. In the context of prostate cancer, red and processed meat[105] and eggs[106] have been shown to increase prostate cancer risk when consumed in higher amounts. There is also substantial evidence that soy foods[17], a common alternative to meat in vegetarian and vegan diets, have a protective effect against prostate cancer. Carotenoids are obtained almost exclusively from plant sources, so increasing intakes of all fruits and vegetables should decrease prostate cancer risk and improve overall morbidity. Increased fruit and vegetable intake would also be beneficial, as intakes of specific fruits and good predictors of certain individual plasma carotenoid levels[107]. Increased plant food from high carotenoid sources such as carrots, cruciferous vegetables (kale, spinach, turnip greens, collards, etc.), tomatoes, pumpkin, squash, tangerines/mandarins/oranges, and peppers should be incorporated into nutrition guidelines for prostate cancer prevention.

5.14. Future Directions: Developing a Prostate Cancer Diet Score (PCDS)

Compiling results of meta-analyses of dietary factors and their influence on prostate cancer would facilitate the creation of a dietary assessment tool. This would be in conjunction with the introduction of a set of dietary guidelines targeting at-risk groups for prostate cancer. Dietary guidelines as a chemopreventative measure or treatment for preclinical prostate cancer would eliminate the burdens of over diagnosis and redundant therapy for non-fatal cases[3]. Adherence to dietary modifications has been shown to be favourable[108, 109], so these guidelines and PCDS would provide a simple, cost-effective, easy-to-use treatment option for low-grade prostate cancer.

More studies of dietary investigations will need to be completed before these guidelines and PCDS can begin to take shape. Possible candidates identified during the initial literature review for this project include allium vegetables, carbohydrates (flour, grains, sugar, etc.), phosphorous, zinc, iron, different fish types, and animal and plant proteins. There are sufficient data available for a meta-analysis of β-carotene, and other carotenoids have also demonstrated anti-cancer mechanisms[110]. Further work is need to determine the influence of retinol and vitamin A, particularly their association with the provitamins in this project. Results of already established analyses would also have to be re-evaluated to quantify ideal intakes.

Some studies have assessed dietary patterns and their effect on prostate cancer by creating specified food matrices and examining overall effects of food intakes[111-113]. One of the studies included in this analysis[64] also developed an oxidative balance score based on the pro- or anti-oxidant effects of different factors on prostate cancer development. Once a sufficient number of relevant dietary factors with significant influence (be it positive or negative) on prostate cancer have been accumulated, their influence will be assessed[114] and quantified, and statistical models can be built to create the PCDS.

6. Conclusion

This meta-analysis of four dietary carotenoids has shown that there is a reduced risk of prostate cancer among men with higher intakes of α-carotene, β-cryptoxanthin, lutein, and zeaxanthin. Increased intakes of high carotenoid foods may be protective against prostate cancer development. Further research and quantification of ideal intake levels is required before recommendations for a prostate cancer diet score can be determined.

Appendix 1 – Results from Most Recent Meta-analyses of Dietary Factors and Prostate Cancer Incidence

Nutrient/Food	Risk Estimate	95% CI	Most Recent Meta-Analysis	Reference
Coffee	0.88	0.82–0.95	2014	Cao[21]
Green Tea	0.79	0.43-1.14		Lin[71]
Black Tea	0.88	0.73-1.02		
Carrots	0.82	0.70-0.97		Xu[24]
ALA	1.08	0.90-1.29	2013	Carleton[115]
Fruit	1.02	0.98-1.07		Meng[116]
Veg	0.97	0.93-1.01		
Raw Tomato	0.81	0.59-1.10	2012	Chen[32]
Cooked Tomato	0.85	0.69-1.06		
Lycopene	0.93	0.86-1.01		
Long Chain n-3 PUFAs	1.06[a]	0.88-1.28		Chua[117]
Arachadonic Acid	1.09[a]	0.97-1.23		
DHA	0.99[a]	0.92-1.07		
EPA	1.00[a]	0.92-1.08		
Linoleic Acid	0.97a	0.86-1.10		
Total Omega 3	0.97a	0.89-1.07		
Total Omega 6	1.04a	0.95-1.13		
Cruciferous Veg	0.90	0.85–0.96		Liu[19]
Alcohol	1.08	0.97–1.20		Rota[118]
Fish	0.85	0.72-1.00		Szymanski[20]
Egg	1.09[b]	0.86-1.31		Xie[106]
Vitamin D	0.83[b]	0.28-2.43	2011	Gilbert[119]
Processed Meat	1.05	0.99-1.12	2010	Alexander[105]
Red Meat	1.00	0.96-1.05		
Daidzein	0.66	0.51-0.86	2009	Hwang[17]
Genistein	0.67	0.52-0.86		
Non-Fermented Soy Food	0.75	0.62-0.89		
Tofu	0.73	0.57-0.92		
Total Soy Food	0.69	0.57-0.84		
Soybean Milk	0.57	0.19-1.76		
Dairy Products	1.11[a]	1.03–1.19	2008	Huncharek[22]
Sat Fat	1.09	0.99-1.20	2004	Dennis[23]
Total Fat	1.17	1.10-1.25		

Statistically significant results highlighted; [a] = figure for cohort studies only, [b] = figure for case-control studies only

Appendix 2 – Details of Initial Literature Review of Dietary Factors and Prostate Cancer

Between January 13th and February 6th a preliminary literature review of dietary factors and prostate cancer was performed using the electronic PubMed (MEDLINE) database. The search terms were "nutrition" OR "diet" AND "prostatic neoplasms", and the search comprised all studies published up to January 2014. Studies were briefly evaluated based on their titles and abstracts. In addition further studies were identified by reviewing the references cited in relevant articles. Laboratory studies and dietary intervention trials were not considered for review. The results of the search are summarised in Figure A below.

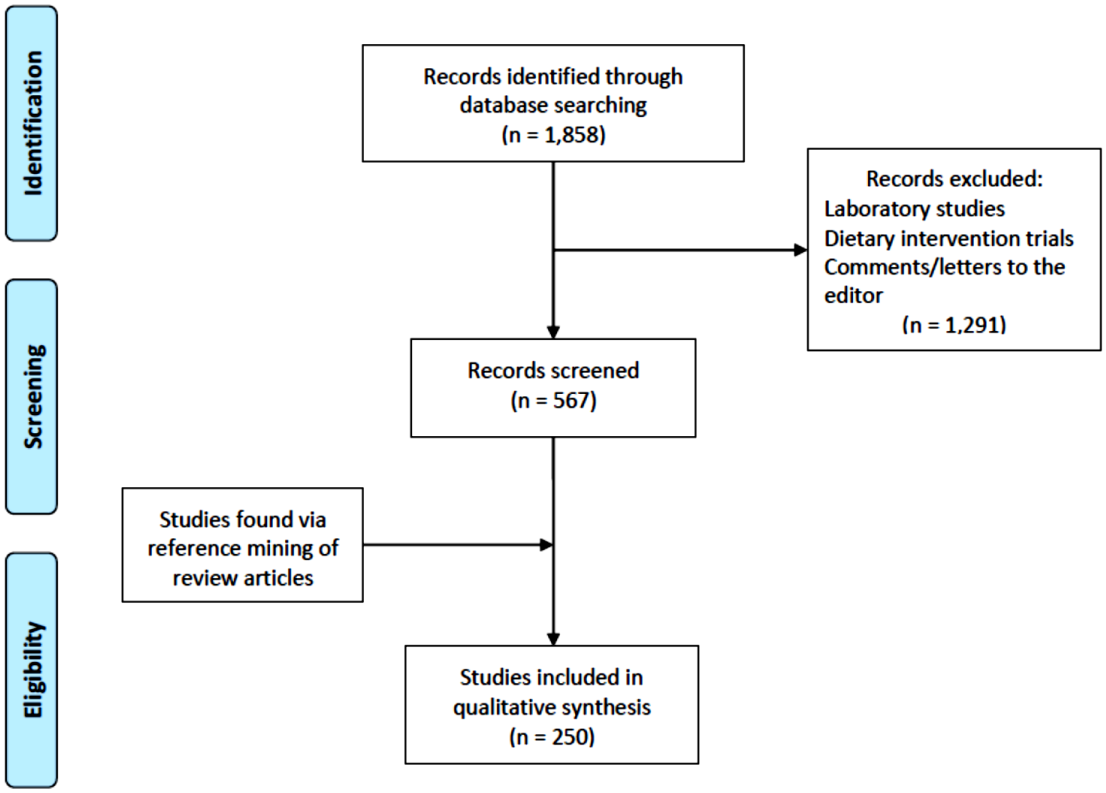

Figure A – PRISMA[54] Flow Diagram Summarising Initial Literature Review of Dietary Factors and Prostate Cancer Incidence

Appendix 3 – Summary of Dietary Assessment Methods Used in Included Studies

Reference	*FFQ*	*Validated*	*Adapted*	*# Items*	*Carotenoid Estimates*	*Questionnaire Self-Administered*	*Interview*	*Timeframe*	*Portion Size*	*Frequency of Consumption*	*Supplement Use*
Agalliu[64]	yes	yes	NCI Canada	166 food items	USDA nutrient database modified to reflect Canadian food availability and nutrient fortification laws.	yes	no	Usual intake over past year	Usual (average) portion size		Multivitamin and single supplement and mineral use - # pills/week, # months of use
Bosetti[56]	yes	yes		78 foods, beverages & recipes	Italian food composition database (Salvini et al)	no	yes	Usual diet during the 2 years prior to cancer diagnosis/hospital admission		Weekly frequency of consumption of each dietary item	
Cohen[59]	yes			99 food items, including 12 fruit items and 21 vegetable items	Incorporated updated data from the USDA on carotenoid content of fruits and vegetables	yes	no	3-5 year period preceding reference dates	3 options for portion size	9 options for frequency	
Deneo-[Pellegrin60i]	yes	no		64	Mangels et al (1993 USA)	no	yes	Past year/year prior to onset of symptoms	A commonly used unit/portion size was specified for each food, open-ended responses	Responses converted to times per year	

Giovannucci[68]	yes	yes		131 food and beverage items, 46 fruit, veg & related items	USDA sources	yes	no	Past year	Commonly used unit or portion size was specified for each food item	9 possible responses	Brand, duration, and frequency of multivitamin and individual vitamin supplement use
Hodge[57]	yes		Developed specifically for use in Australian epidemiological studies	121 item FFQ - 29 groups (some subgroups of others)	Version 11 of the USDA carotenoid database	no	yes				
Jain[61]	no	yes	Quantitative diet history encompassing 1,129 unique food items	Classified into 29 food groups for analysis	USDA-National Cancer Institute carotenoid food composition database	no	yes	One year prior to diagnosis/interview date			
Jian58	yes	yes	Modified from FFQs from 4 other sources	130 food items	USDA nutrient database	no	yes	5 years before diagnosis/interview		Options ranging from 0-2 times/year to ≥2 times/day	
Kirsh[65]	yes			137 food items	University of Minnesota Nutrition Data System for Research	yes	no	Previous year	Usual portion size (S/M/L)		Multivitamins & single-nutrient supplements - duration, frequency, dose/day, when they started taking them
Lu[51]	yes		NCI HHHQ short FFQ		Nutrient contents calculated using an NCI algorithm, based on USDA database	no	yes	One year prior to diagnosis/interview date	Usual dietary patterns, usual portion size	Frequency of consumption	

McCann[55]	yes	no	Comparable to FFQs used by NCI & Harvard NHS	172	Food composition data from USDA	no	yes	2 years prior to interview	Included info on portion size		
Meyer[62]	yes	yes	Food list modified to better reflect the dietary habits of the French-speaking population of Quebec, and expanded to improve the dietary assessment of fat, retinol, and carotenoid intake	143 foods or dishes	Mangels et al 1993, micronutrient intake from supplements was computed using the 1993 Canadian Compendium of Pharmaceutical Specialties	no	yes	Previous 12 months	Three-dimensional models were used to determine portion size	Intake frequency	Intake of vitamin and mineral supplements, name and amount of each supplement, frequency and duration of use
Rohrmann[48]	yes	yes		131 item semi-quantitative	USDA sources	yes	no		Commonly used unit or serving size specified for each food item	9 possible response categories ranging from “never” to “6 or more times per day”	Dose and duration of vitamin supplement intake
Schuurman[67]	yes	yes		150 items semi-quantitative FFQ	Goldbohm et al 1988	yes	no	Year preceding start of study		Habitual consumption	Any vitamin supplement usage during five years before baseline

Stram[66]	yes		180	USDA nutrient database	yes	no	Previous year	Photographs of representative food items showing three different portion sizes were used to facilitate quantification of intakes	Frequency of consumption	.
Umesawa[63]	yes	yes	35 foods		yes	no			5 responses were possible ranging from 'rarely', to 'almost every day'	

Appendix 4 – Exposure Assessment Boundaries and Adjusted Covariates

4.1. α-Carotene

Reference	Adjustments	Measurement	Q1	Q2	Q3	Q4	Q5
Bosetti[56]	age, study centre, education, physical activity, body mass index, family history of prostate cancer and total calorie intake	µg, mean values (SD)	not reported				
Cohen[59]	fat, energy, race, age, family history of prostate cancer, body mass index, prostate-specific antigen tests in previous 5 years, and education	µg	<330	330-549	550-809	≥810	
Deneo-Pellegrini[60]	age, residence, urban/rural status, education, family history of prostate cancer, body mass index and total energy intake	µg/day	≤109	110–291	292–600	601+	
Giovannucci[68]	energy-adjusted nutrient, adjusted for age by stratified analysis	µg	<380	380-522	523-722	723-1339	>1339
Hodge[57]	state, age group, year, country of birth, socioeconomic group, and family history of prostate cancer	µg/day	670-1243	1244-1497	1498-2125	2126+	
Jain[61]	log total energy, vasectomy, age, ever-smoked, marital status, study area, body mass index, education, ever-used multivitamin supplements in 1 yr. before diagnosis/interview, area of study, and log-converted amounts for grains, fruit, vegetables, total plants, total carotenoids, folic acid, dietary fibre, conjugated linoleic acid, vitamin E, vitamin C, retinol, total fat, and linoleic acid	µg/day	<839	839-1514	1515-2187	>2158	
Jian[58]	age at interview, BMI, locality of residence, education, family income, marital status, number of children, family history of prostate cancer, tea drinking, total caloric intake (kcal/day) and total fat intake (gm/day)	µg/day	<238.9	238.9-747.5	747.6-1786	>1786	
Kirsh[65]	age, total energy, race, study centre, family history of prostate cancer, body mass index, smoking status, physical activity, total fat intake, red meat intake, history of diabetes, aspirin use, and number of screening examinations during	median µg/day	472	784	1081	1476	2317

	the follow-up period						
Lu[51]	age, race, education, alcohol drinking, pack-years of smoking, family history of prostate cancer, and total dietary caloric intake	µg	<385.765	385.766-699.293	699.294-1142.31	>1142.32	
McCann[55]	age, education, body mass index, cigarette smoking status, total energy, veg intake	µg/day	≤626	626-977	977-1488	>1488	
Meyer[62]	age, education, family history of prostate cancer, group, dietary energy						
Rohrmann[48]	age, race or ethnicity, cigarette smoking, BMI, leisure-time physical activity, alcohol consumption, energy intake, intake of protein, and intake of polyunsaturated fatty acids	µg/day	293	493	634	1019	2040
Schuurman[67]	age, family history of prostate cancer, socioeconomic status, and alcohol from white or fortified wine	mg/day	0,2	0,4	0,6	0,8	1,3
Stram[66]	age, BMI, education and family history of prostate cancer	µg/1000kcal	≤170.8	170.9-264.2	264.3-382.7	382.8-623	≥623.1
Umesawa[63]	age, BMI, ethanol intake, smoking status, daily green tea intake and work schedule	median µg/day	105	175	236	317	496

4.2. β-Cryptoxanthin

Reference	Adjustments	Measurement	Q1	Q2	Q3	Q4	Q5
Agalliu[61]	age at baseline, race, BMI, exercise activity, and education	µg, median values	83.3	164.5	211.3	269.1	388.2
Bosetti[56]	age, study centre, education, physical activity, body mass index, family history of prostate cancer and total calorie intake	µg, mean values (SD)	Not reported				
Cohen[59]	fat, energy, race, age, family history of prostate cancer, body mass index, prostate-specific antigen tests in previous 5 years, and education	µg	<10	10-24	25-44	≥45	
Giovannucci[68]	energy-adjusted nutrient, adjusted for age by stratified analysis	µg	<22	22-40	41-67	68-114	>114
Hodge[57]	state, age group, year, country of birth, socioeconomic group, and family history of prostate cancer	µg/day	152-272	273-415	416-657	658+	
Jain[61]	log total energy, vasectomy, age, ever-smoked, marital status, study area, body mass index, education, ever-used multivitamin supplements in 1 year before diagnosis/interview, area of study, and log-converted amounts for grains, fruit, vegetables, total plants, total carotenoids, folic acid, dietary fibre, conjugated linoleic acid, vitamin E, vitamin C, retinol, total fat, and linoleic acid	µg/day	<17.9	17.9-49.4	49.5-100	>100	
Jian[58]	age at interview, BMI, locality of residence,	µg/day	<70.7	70.7-126.8	126.9-230.3	>230.3	

	education, family income, marital status, number of children, family history of prostate cancer, tea drinking, total caloric intake (kcal/day) and total fat intake (gm/day						
Kirsh[65]	age, total energy, race, study centre, family history of prostate cancer, body mass index, smoking status, physical activity, total fat intake, red meat intake, history of diabetes, aspirin use, and number of screening examinations during the follow-up period	median µg/day	65	122	178	241	359
Lu[51]	age, race, education, alcohol drinking, pack-years of smoking, family history of prostate cancer, and total dietary caloric intake	µg	<23.0516	23.0517-71.1566	71.1567-120.847	>120.848	
McCann[55]	age, education, body mass index, cigarette smoking status, total energy, veg intake	µg	≤36	36-99	99-201	>201	
Rohrmann[48]	age, race or ethnicity, cigarette smoking, BMI, leisure-time physical activity, alcohol consumption, energy intake, intake of protein, and intake of polyunsaturated fatty acids	µg	11	33	56	93	171
Schuurman[67]	age, family history of prostate cancer, socioeconomic status, and alcohol from white or fortified wine	mg/day	0.012	0.045	0.1	0.2	0.4
Stram[66]	age, BMI, education and family history of prostate cancer	µg/1000kcal	≤19.5	19.6-48.1	48.2-91.7	91.8-189.9	≥190

4.3. Lutein

Reference	Adjustments	Measurement	Q1	Q2	Q3	Q4	Q5
Deneo-Pellegrini[60]	age, residence, urban/rural status, education, family history of prostate cancer, body mass index and total energy intake	µg/day	≤1214	1215–2086	2087–3593	3594+	
Giovannucci[68]	energy-adjusted nutrient, adjusted for age by stratified analysis	µg	<1799	1799-2665	2666-3620	3621-5100	>5100
Jain[61]	log total energy, vasectomy, age, ever-smoked, marital status, study area, body mass index, education, ever-used multivitamin supplements in 1 yr. before diagnosis/interview, area of study, and log-converted amounts for grains, fruit, vegetables, total plants, total carotenoids, folic acid, dietary fibre, conjugated linoleic acid, vitamin E, vitamin C, retinol, total fat, and linoleic acid	µg/day	<1019	1018-1653	1654-2684	>2684	
Lu[51]	age, race, education, alcohol drinking, pack-years of smoking, family history of prostate cancer, and total dietary caloric intake	µg	<1009.78	1009.79-1666.75	1666.76-2916.75	>2916.76	
McCann[55]	age, education, body mass index, cigarette smoking status, total energy, veg intake	µg/day	3029	3029-4975	4975-7168	>7168	
Meyer[62]	age, education, family history of prostate cancer, group, dietary energy	not reported					
Stram[66]	age, BMI, education and family history of prostate cancer	µg/1000kcal	≤594.4	594.5-852.7	852.7-1158.2	1158.3-1661.3	≥1661.4

4.4. Lutein and Zeaxanthin

Reference	Adjustments	Measurement	Q1	Q2	Q3	Q4	Q5
Agalliu[64]	age at baseline, race, BMI, exercise activity, and education	µg, median values	1617.5	2220.1	2763.4	3506.2	5346.0
Bosetti[56]	Estimates from multiple logistic regression models including terms for age, study centre, education, physical activity, body mass index, family history of prostate cancer and total calorie intake	µg, mean values (SD)					
Cohen[59]	fat, energy, race, age, family history of prostate cancer, body mass index, prostate-specific antigen tests in previous 5 years, and education	µg	<800	800-1299	1300-1999	≥2000	
Hodge[57]	state, age group, year, country of birth, socioeconomic group, and family history of prostate cancer	µg/day	1101-1531	1532-1891	1892-2456	2457+	
Jian[58]	age at interview, BMI, locality of residence, education, family income, marital status, number of children, family history of prostate cancer, tea drinking, total caloric intake (kcal/day) and total fat intake (gm/day	µg/day	<746.2	746.2-1718.4	1718.5-3590.6	>3590.6	
Kirsh[65]	age, total energy, race, study centre, family history of prostate cancer, body mass index, smoking status, physical activity, total fat intake, red meat intake, history of diabetes, aspirin use, and number of screening examinations during the follow-up period	median µg/day	1437	1995	2501	3138	4428
Rohrmann[48]	age, race or ethnicity, cigarette smoking, BMI, leisure-time physical activity, alcohol consumption, energy intake, intake of protein, and intake of polyunsaturated fatty acids	µg/day	1308	2271	3184	4347	6788
Schuurman[67]	age, family history of prostate cancer, socioeconomic status, and alcohol from white or fortified wine	mg/day	1.4	1.9	2.4	2.9	3.9

Appendix 5 – Contents of Do Files Used to Complete Meta-analysis in Stata

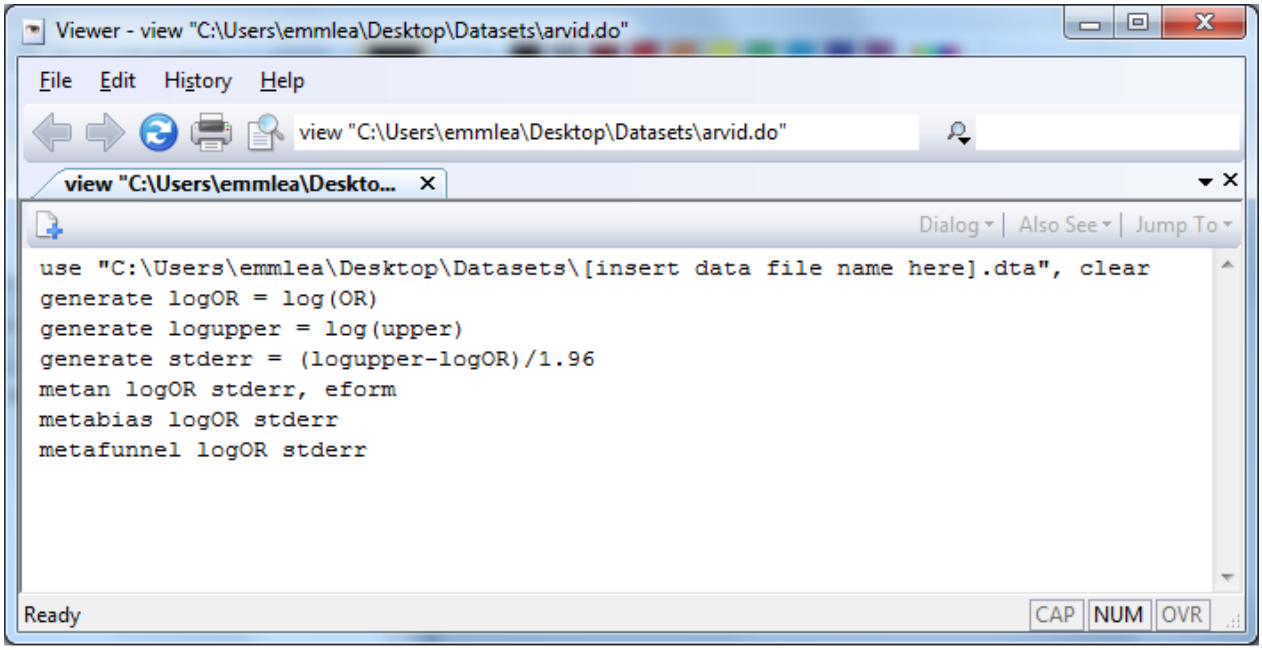

Figure B - Do File Used for Case-control/NCC study analysis

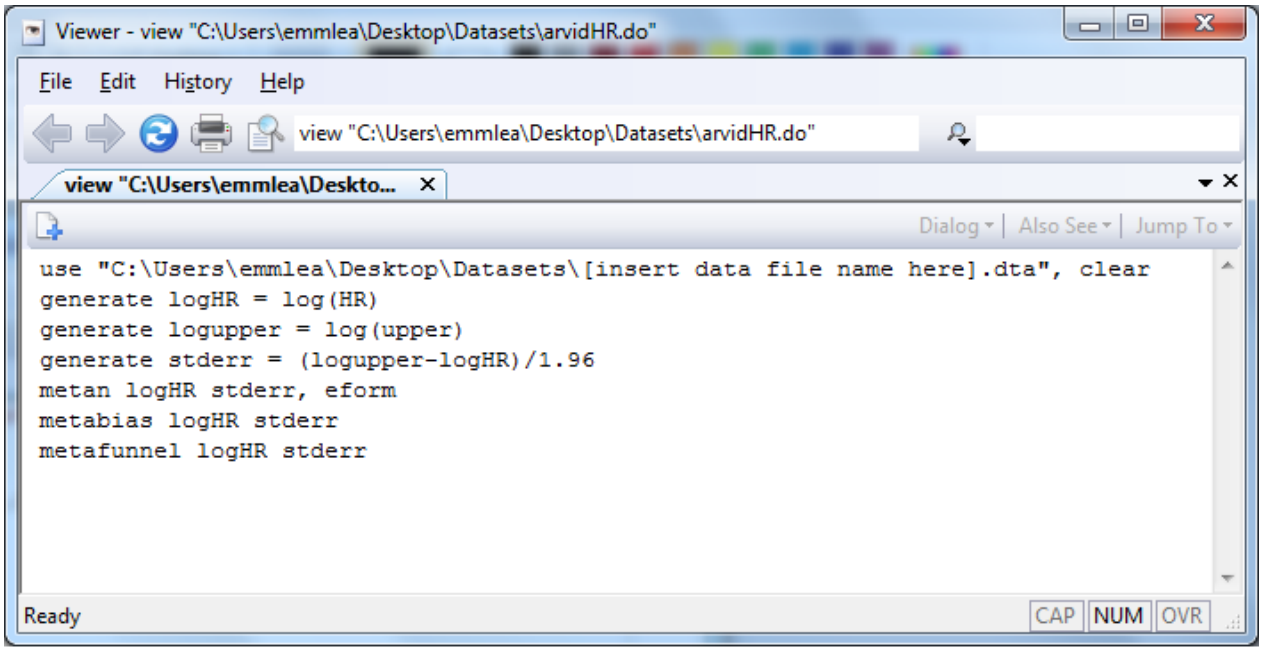

Figure C - Do File Used for Cohort/Case-cohort Study Analysis

Appendix 6 – Full Results of Meta-Analyses of Dietary Intake of Carotenoids and Prostate Cancer Incidence

6.1. α-Carotene

Table A - Full Results of Included Case-Control/NCC Studies

Reference	OR	95% CI	Log OR	SE	Weight (%)
Bosetti[56]	0.85	0.66-1.11	-0.1625189	0.1361627	9.96
Cohen[59]	0.75	0.51–1.09	-0.2876821	0.1907448	5.07
Deneo-Pellegrini[60]	0.9	0.5-1.6	-0.1053605	0.2935531	2.14
Hodge[57]	0.8	0.6-1.1	-0.2231435	0.1624764	6.99
Jain[61]	1.06	0.79-1.43	0.0582689	0.1527579	7.91
Jian[58]	0.43	0.21-0.85	-0.8439701	0.3476792	1.53
Lu[51]	0.47	0.14–1.66	-0.7550226	0.643796	0.45
McCann[55]	0.91	0.59-1.39	-0.0943106	0.2161298	3.95
Meyer[62]	1.00	0.53-1.89	0	0.3247841	1.75
Rohrmann[48]	0.96	0.87-1.07	-0.040822	0.0553473	60.25
Overall	0.915	0.841-0.996	-0.088831	-	100

Heterogeneity	**Chi^2 (d.f.)**	**p**	**I^2 (%)**
	9.61 (9)	0.383	6.3
Test of Overall Effect	**z**	**p**	
	2.06	0.039	

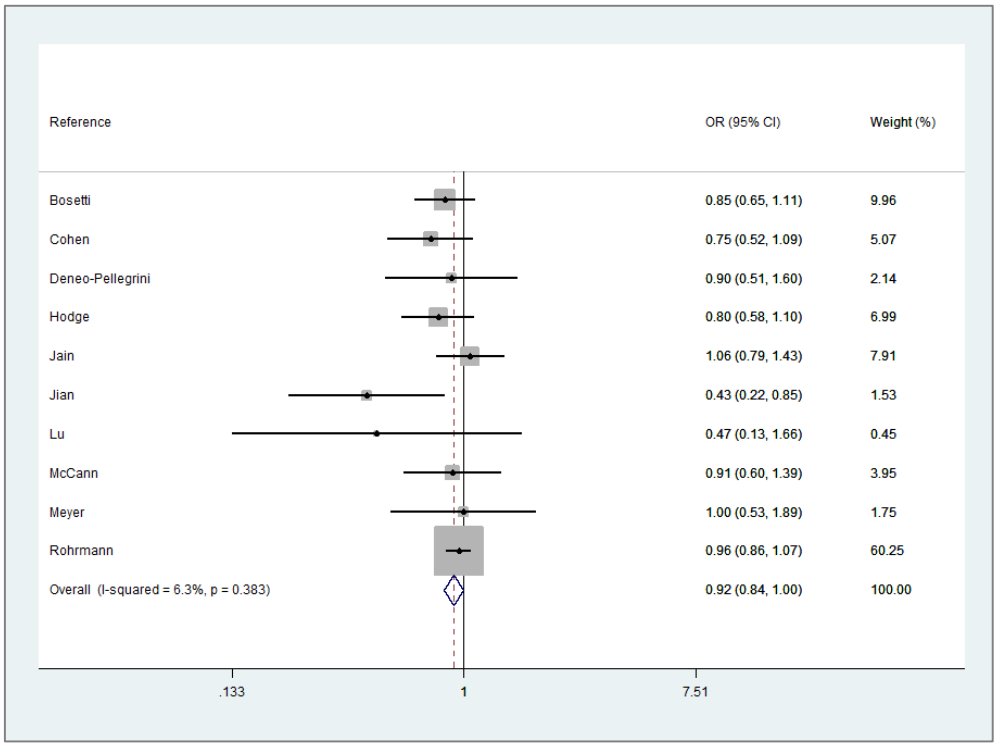

Figure D - Forrest Plot Showing Risk Estimates from Included Case–Control/NCC Studies of Dietary α-Carotene Intake and Prostate Cancer Risk

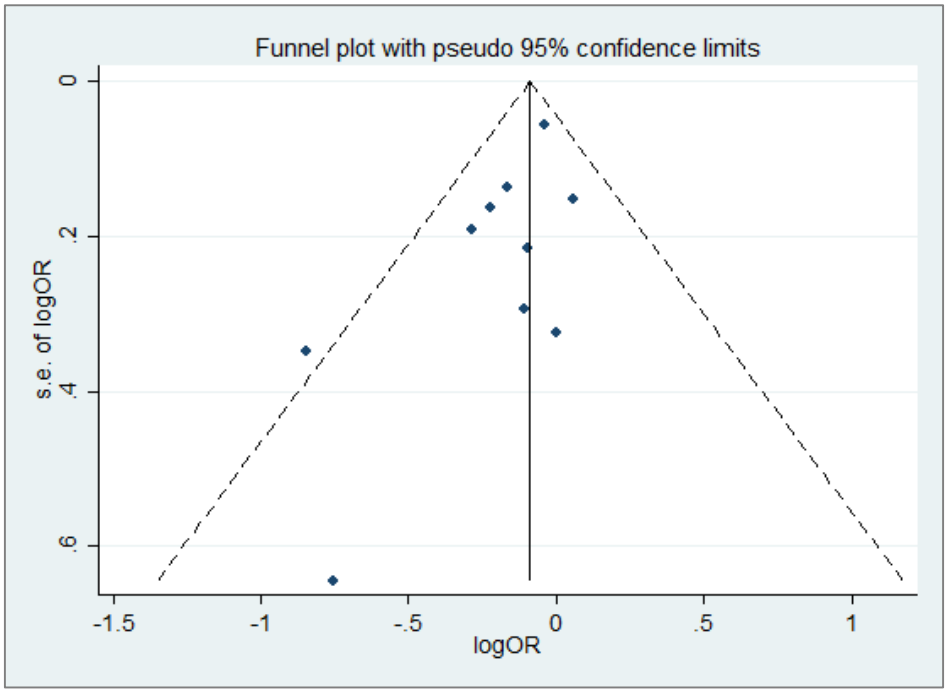

Figure E - Funnel Plot Examining Publication Bias in Included Case-Control/NCC Studies of Dietary α-Carotene and Prostate Cancer Risk

Table B - Full Results of Included Cohort/Case-Cohort Studies

Reference	HR	95% CI	log HR	SE	Weight (%)
Giovannucci[68]	1.09	0.87-1.36	0.0861777	0.1129117	12.56
Kirsh[65]	0.92	0.76-1.10	-0.0833816	0.0911693	19.26
Schuurman[67]	0.85	0.62-1.17	-0.1625189	0.1630217	6.02
Stram[66]	0.94	0.85-1.04	-0.0618754	0.0515796	60.17
Umesawa[63]	0.74	0.42–1.29	-0.3011051	0.2835445	1.99
Overall	0.943	0.872-1.020	-0.058689	-	100

Heterogeneity	**Chi^2 (d.f.)**	**p**	**I^2 (%)**
	2.86 (4)	0.582	0.0
Test of Overall Effect	**z**	**p**	
	1.46	0.145	

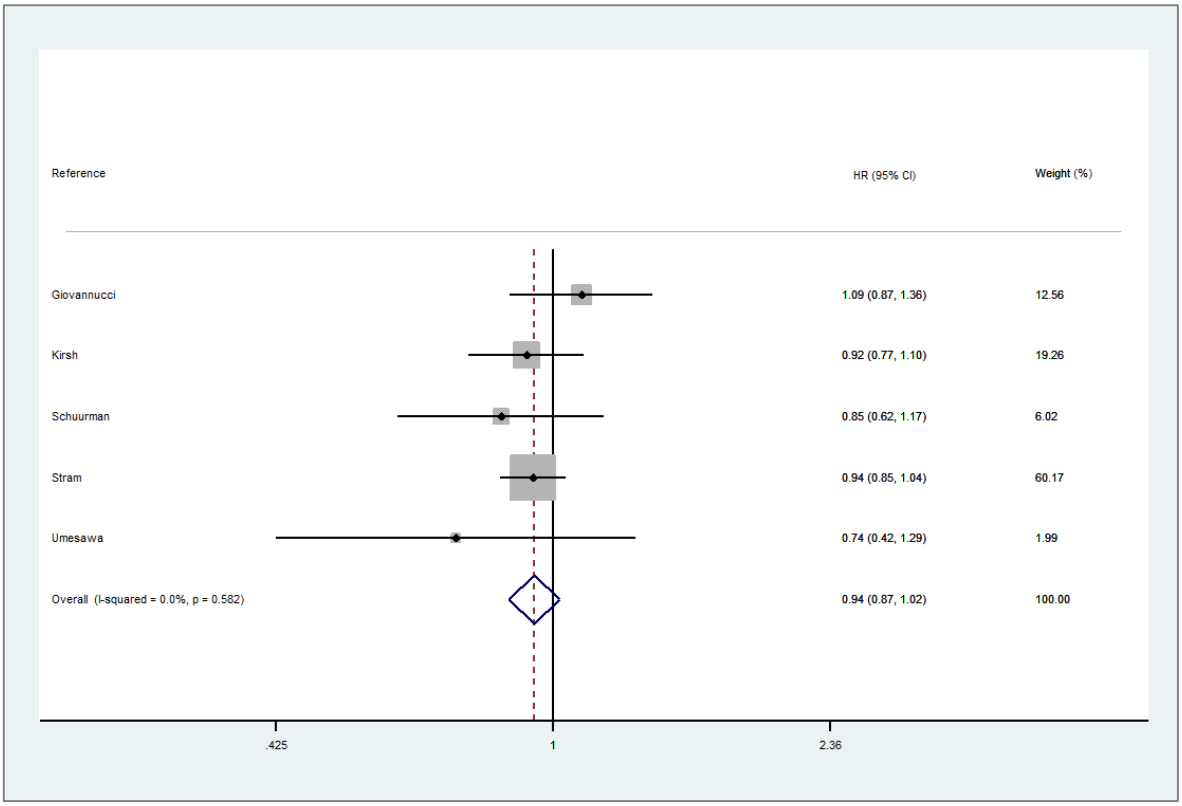

Figure F - Forrest Plot Showing Risk Estimates from Included Cohort/Case-Cohort Studies of Dietary α-Carotene Intake and Prostate Cancer Risk

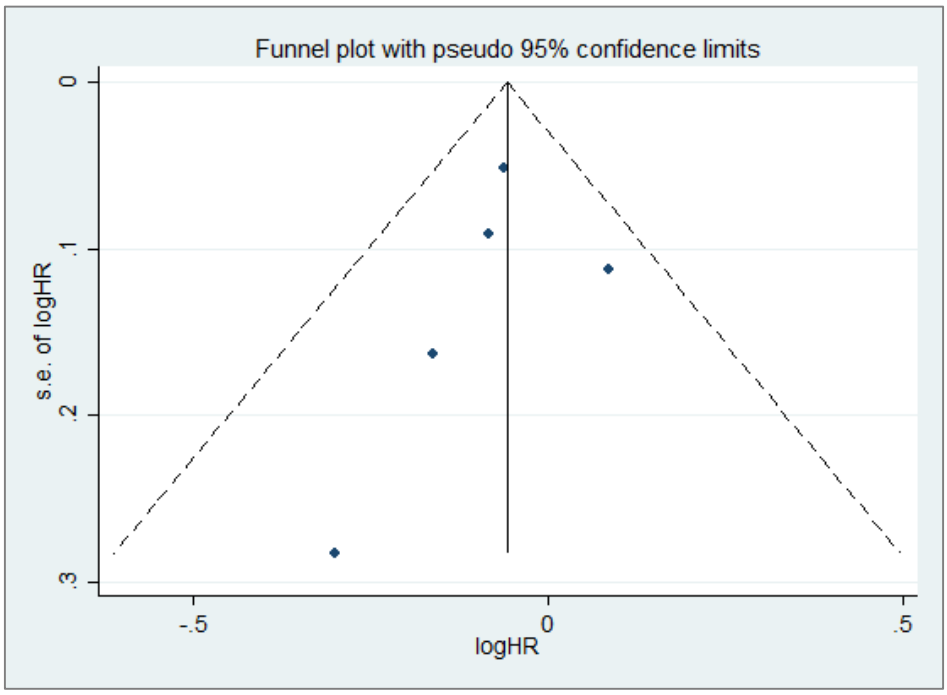

Figure G - Funnel Plot Examining Publication Bias in Included Cohort/Case-Cohort Studies of Dietary α-Carotene and Prostate Cancer Risk

6.2. β-Cryptoxanthin

Table C - Full Results of Included Case-Control/NCC Studies

Reference	OR	95% CI	Log OR	SE	Weight (%)
Bosetti[56]	0.9	0.69–1.16	-0.1053605	0.1294799	11.31
Cohen[59]	0.93	0.64-1.36	-0.0725707	0.1939058	5.04
Hodge[57]	0.9	0.7–1.3	-0.1053605	0.1876147	5.38
Jain[61]	1.44	1.09-1.89	0.3646432	0.1387417	9.85
Jian[58]	0.15	0.06-0.34	-1.89712	0.4175053	1.09
Lu[51]	0.92	0.26-3.2	-0.0833816	0.6359859	0.47
McCann[55]	0.92	0.64-1.33	-0.0833816	0.1880411	5.36
Rohrmann[48]	0.87	0.79-0.97	-0.1392621	0.0555117	61.51
Overall	0.908	0.834-0.989	-0.096511	-	100

Heterogeneity	**Chi² (d.f.)**	**p**	**I² (%)**
	30.27	0.000	76.9
Test of Overall Effect	**z**	**p**	
	2.22	0.027	

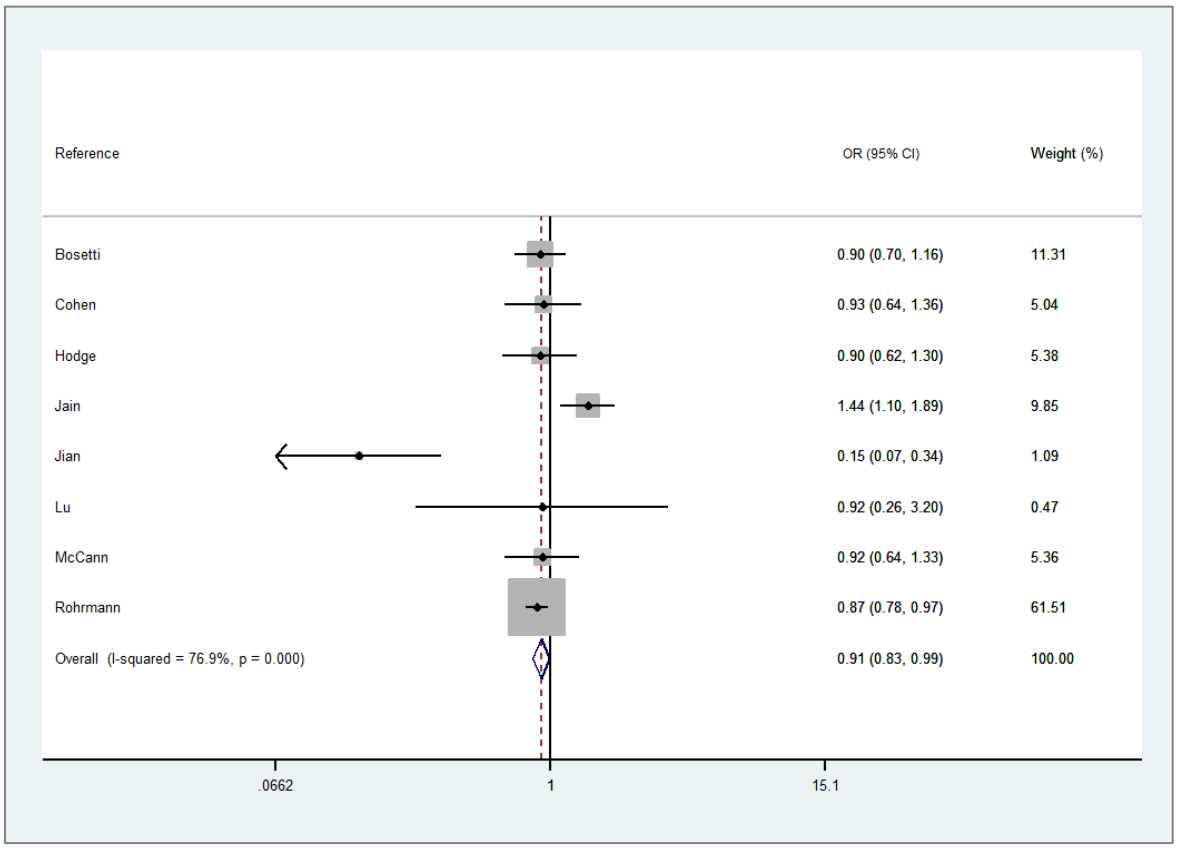

Figure H - Forrest Plot Showing Risk Estimates from Included Case–Control/NCC Studies of Dietary β-Cryptoxanthin Intake and Prostate Cancer Risk

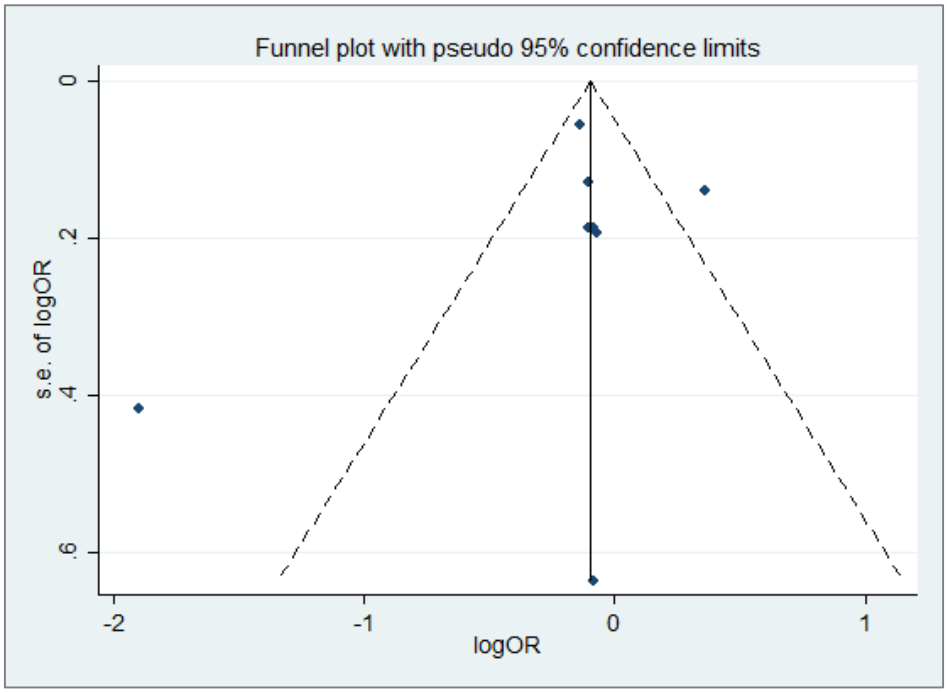

Figure I - Funnel Plot Examining Publication Bias in Included Case-Control/NCC Studies of Dietary β-Cryptoxanthin and Prostate Cancer Risk

Table D - Full Results of Included Cohort/Case-Cohort Studies

Reference	HR	95% CI	Log HR	SE	Weight (%)
Agalliu[64]	0.94	0.69–1.28	-.0618754	0.1575181	6.27
Giovannucci[68]	0.94	0.75-1.17	-.0618754	0.111673	12.47
Kirsh[65]	1.05	0.87-1.27	.0487901	0.0970545	16.51
Schuurman[67]	1.41	1.03-1.92	0.3435897	0.1575181	6.27
Stram[66]	0.94	0.85-1.04	-.0618754	0.0515796	58.47
Overall	0.982	0.909-1.061	-0.018163	-	100

Heterogeneity	**Chi^2 (d.f.)**	**p**	**I^2 (%)**
	6.70	0.153	40.3

Test of Overall Effect	**z**	**p**
	0.46	0.645

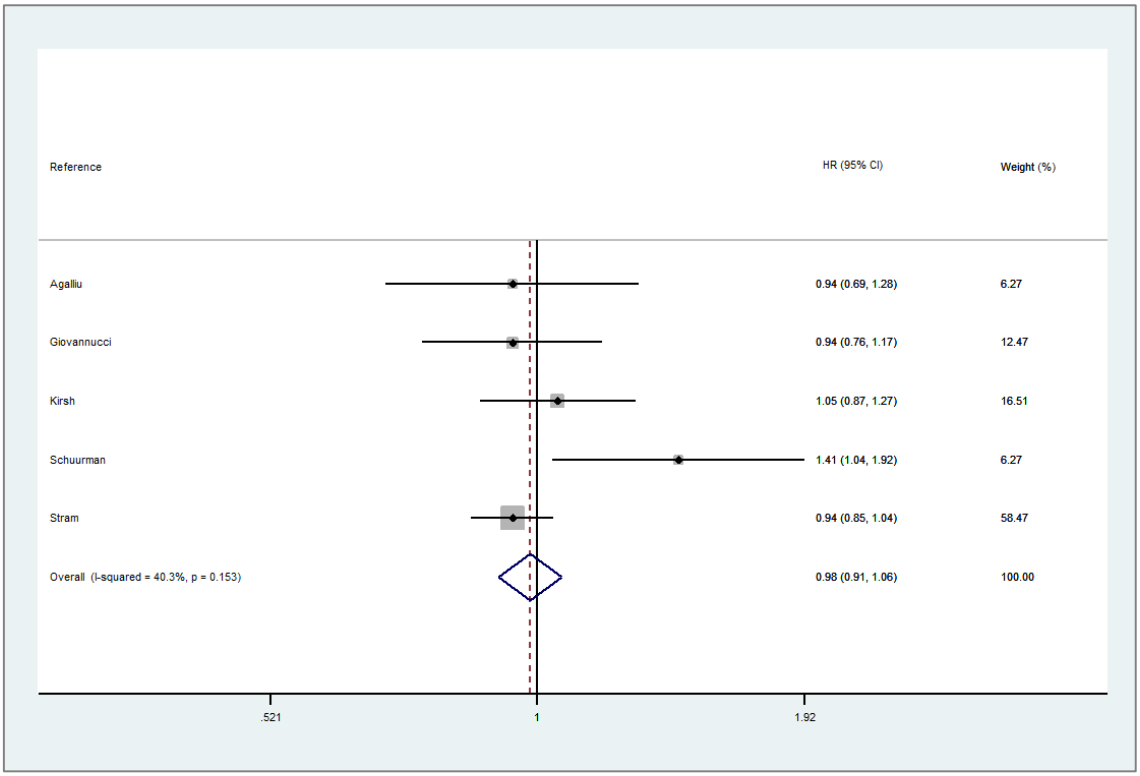

Figure J - Forrest Plot Showing Risk Estimates from Included Cohort/Case-Cohort Studies of Dietary β-Cryptoxanthin Intake and Prostate Cancer Risk

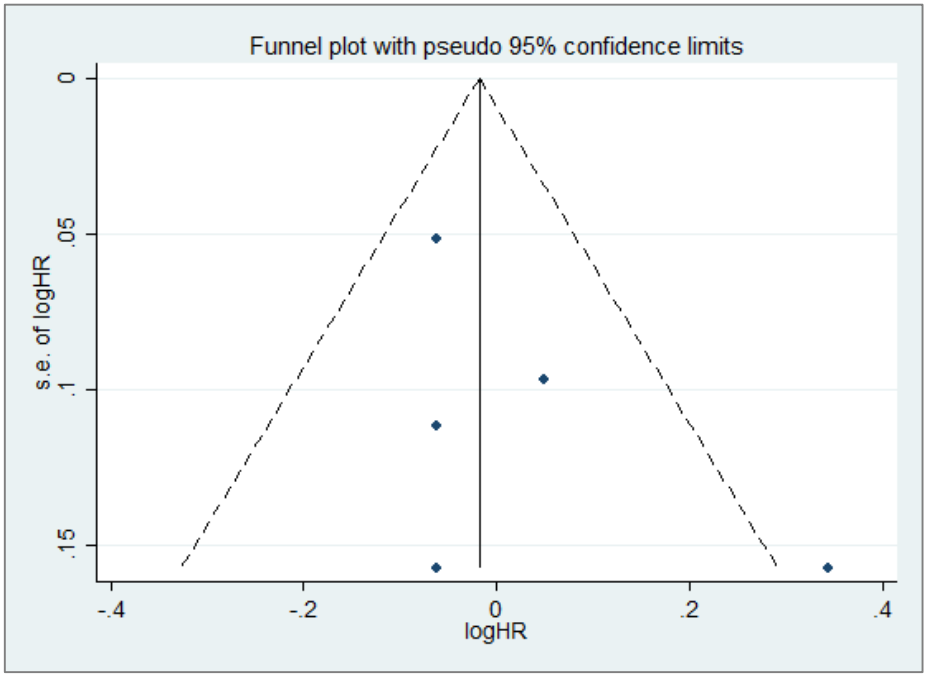

Figure K - Funnel Plot Examining Publication Bias in Included Cohort/Case-Cohort Studies of Dietary β-Cryptoxanthin and Prostate Cancer Risk

6.3. Lutein

Table E - Full Results of Included Case-Control/NCC Studies

Reference	OR	95% CI	log OR	SE	Weight (%)
Deneo-Pellegrini[60]	0.7	0.4-1.3	-0.356675	0.3158363	15.68
Jain[61]	0.81	0.65-1.18	-0.210721	0.1919568	42.46
Lu[51]	0.55	0.16-1.88	-0.597837	0.6270963	3.98
McCann[55]	0.71	0.43-1.16	-0.3424903	0.2504644	24.94
Meyer[62]	0.86	0.44-1.7	-0.1508229	0.3476792	12.94
Overall	0.760	0.595-0.971	-2.744367	-	100

Heterogeneity	**Chi² (d.f.)**	**p**	**I² (%)**
	0.64 (4)	0.958	0.0

Test of Overall Effect	**z**	**p**
	2.19	0.028

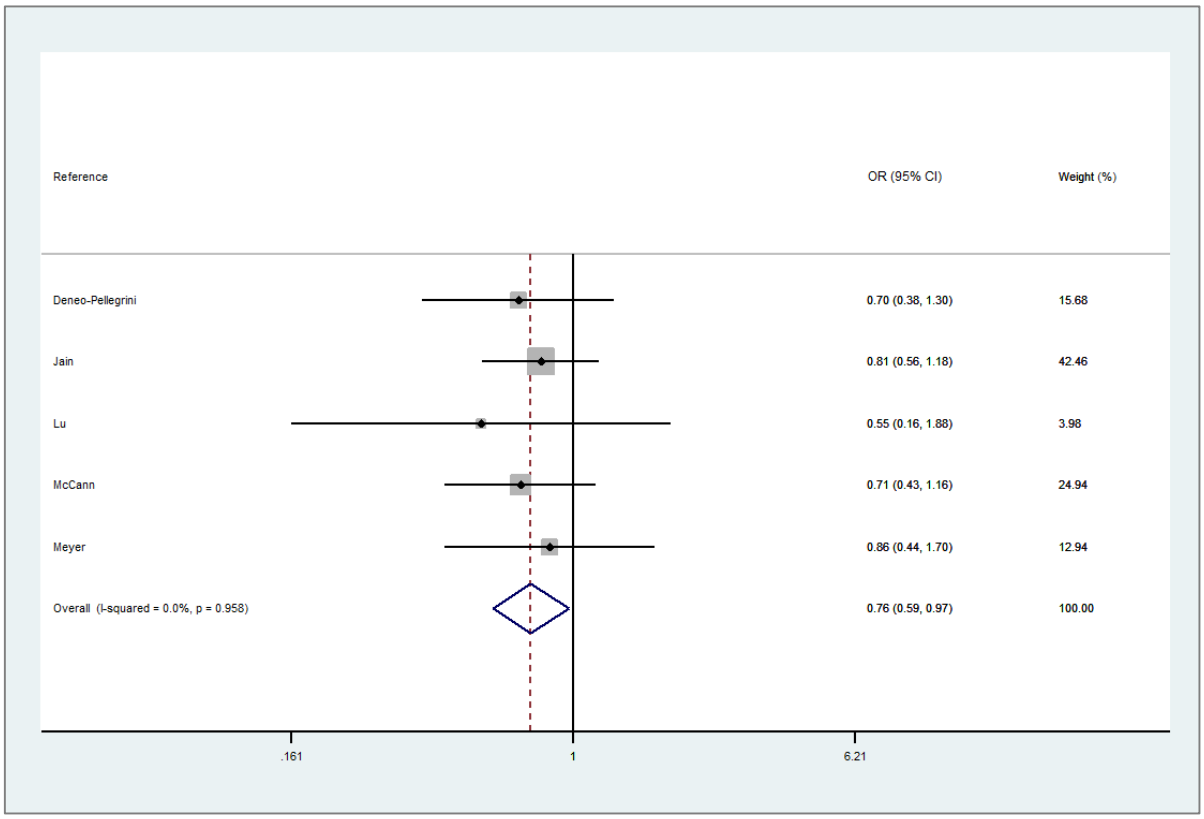

Figure L - Forrest Plot Showing Risk Estimates from Included Case–Control/NCC Studies of Dietary Lutein Intake and Prostate Cancer Risk

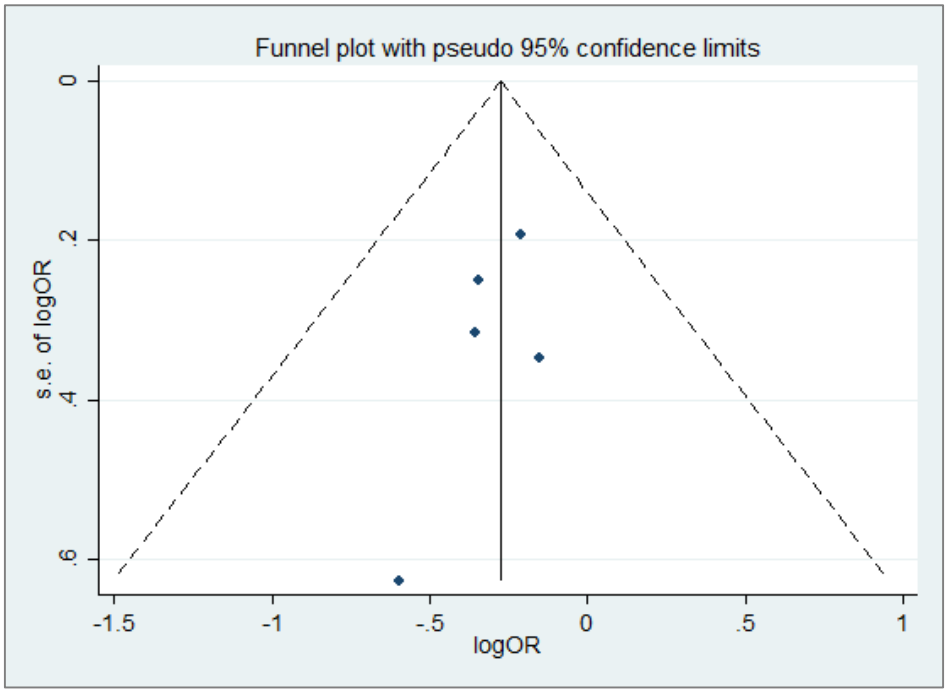

Figure M - Funnel Plot Examining Publication Bias in Included Case-Control/NCC Studies of Lutein and Prostate Cancer Risk

Table F - Full Results of Included Cohort/Case-Cohort Studies

Reference	HR	95% CI	log HR	SE	Weight (%)
Giovannucci[68]	1.1	0.88-1.37	0.0953102	0.1119901	19.02
Stram[66]	0.98	0.88–1.09	-0.0202027	0.0542757	80.98
Overall	1.002	0.910-1.102	0.001998	-	100

Heterogeneity	**Chi² (d.f.)**	**P**	**I² (%)**
	0.86 (1)	0.353	0.0

Test of Overall Effect	**z**	**P**
	0.04	0.971

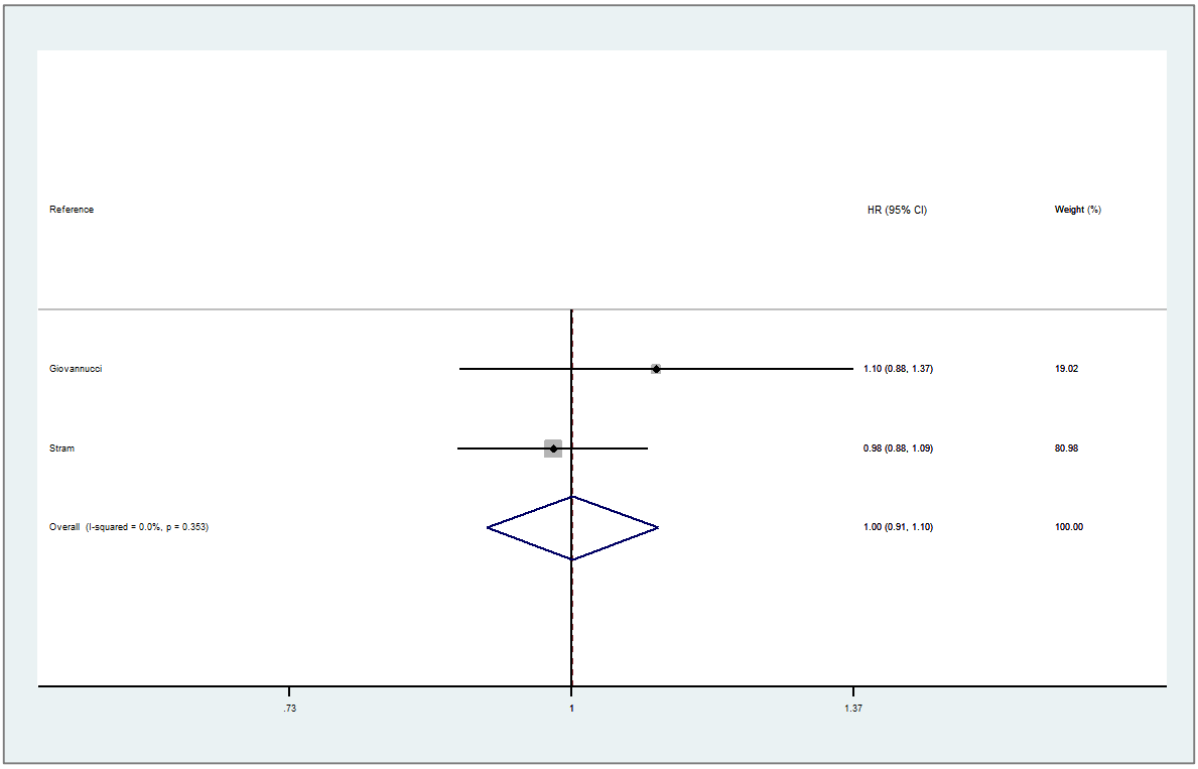

Figure M - Forrest Plot Showing Risk Estimates from Included Cohort/Case-Cohort Studies of Dietary Lutein Intake and Prostate Cancer Risk

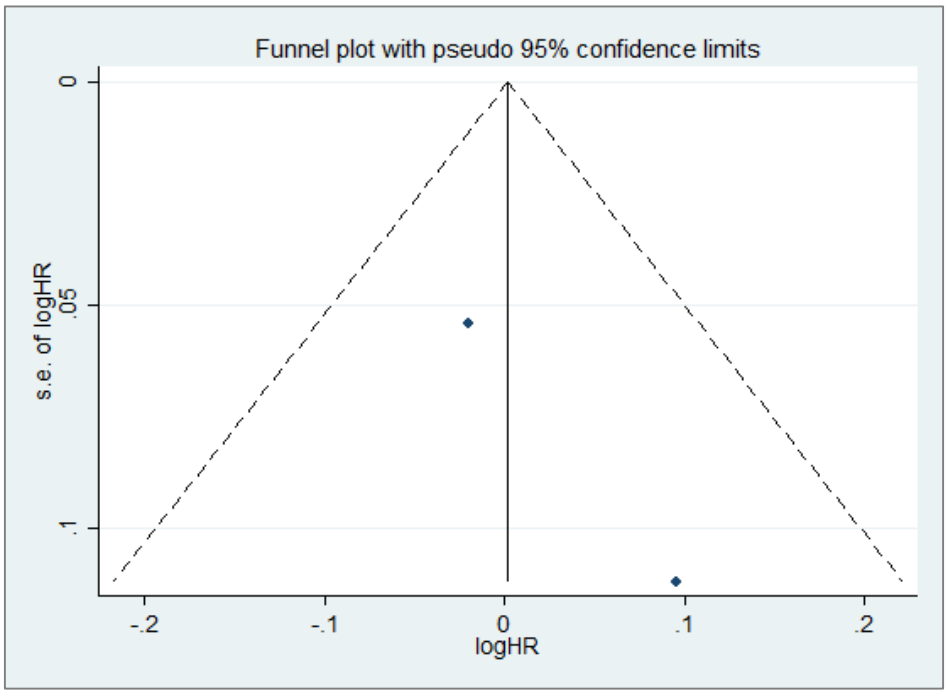

Figure N - Funnel Plot Examining Publication Bias in Included Cohort/Case-Cohort Studies of Dietary Lutein and Prostate Cancer Risk

6.4. Lutein & Zeaxanthin

Table G - Full Results of Included Case-Control/NCC Studies

Reference	OR	95% CI	log OR	SE	Weight (%)
Bosetti[56]	0.91	0.69-1.2	-0.0943106	0.1411389	9.14
Cohen[59]	0.68	0.45-1	-0.3856625	0.1967666	4.70
Hodge[57]	0.9	0.7-1.3	-0.1053605	0.1876147	5.17
Jian[58]	0.02	0.01-0.1	-3.912023	0.8211418	0.27
Rohrmann[48]	0.82	0.74-0.9	-0.198451	0.0474951	80.71
Overall	0.816	0.751-0.888	-0.203341	-	100

Heterogeneity	Chi^2 (d.f.)	p	I^2 (%)
	22.14 (4)	0.000	81.9

Test of Overall Effect	Z	p
	4.76	0.000

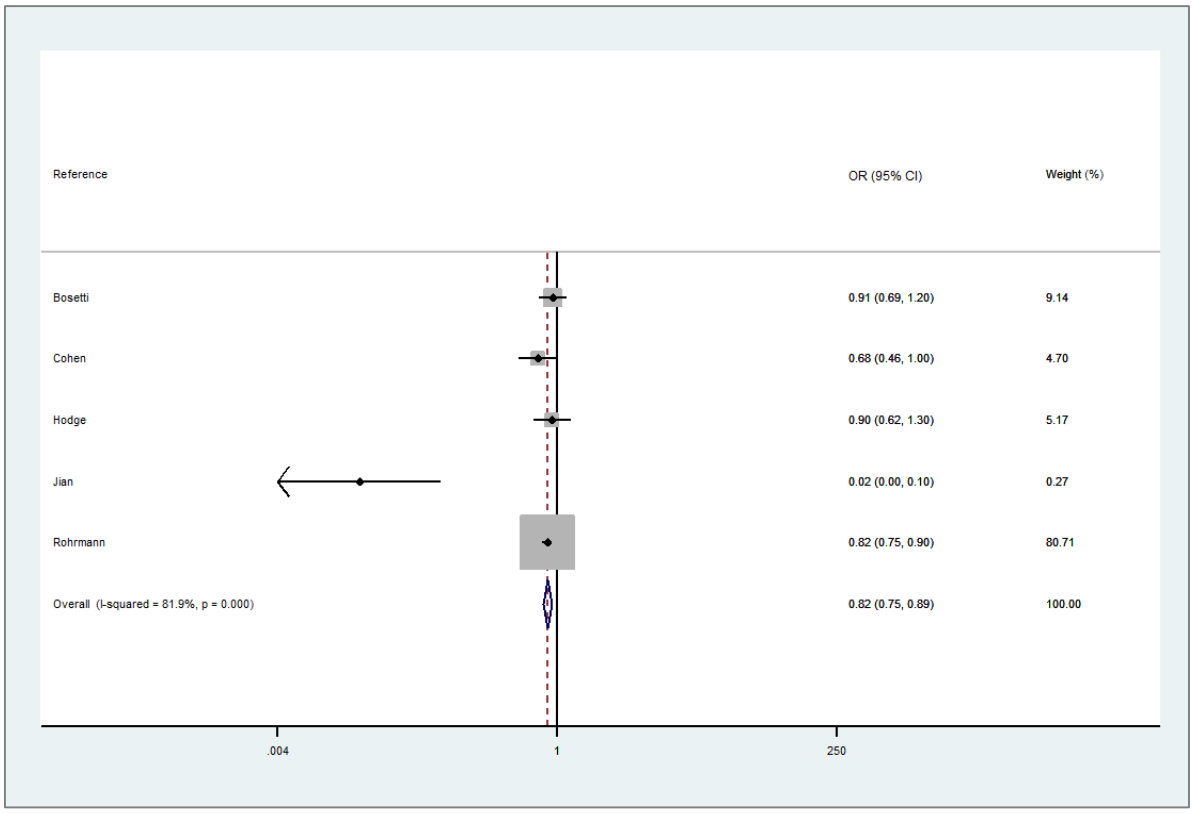

Figure O - Forrest Plot Showing Risk Estimates from Included Case–Control/NCC Studies of Dietary Lutein & Zeaxanthin Intake and Prostate Cancer Risk

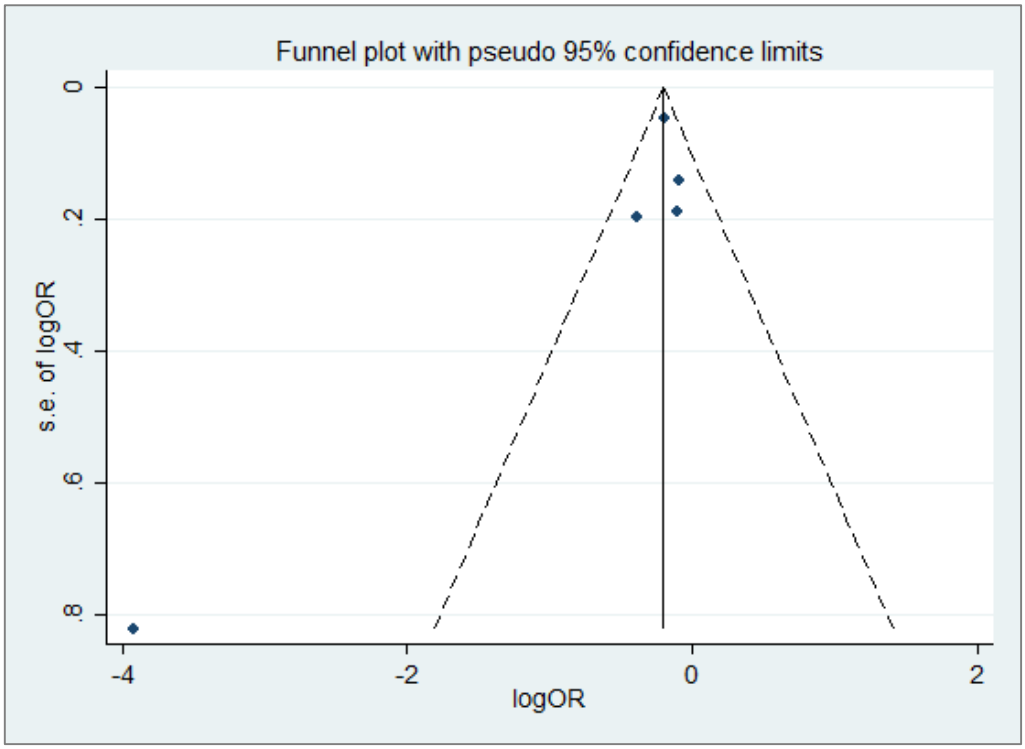

Figure P - Funnel Plot Examining Publication Bias in Included Case-Control/NCC Studies of Dietary Lutein & Zeaxanthin and Prostate Cancer Risk

Table H - Full Results of Included Cohort/Case-Cohort Studies

Reference	HR	95% CI	log HR	SE	Weight (%)
Agalliu[64]	0.97	0.72-1.3	-0.0304592	0.1493997	22.35
Kirsh[65]	0.95	0.78-1.14	-0.0512933	0.0930212	57.64
Schuurman[67]	0.91	0.66-1.24	-0.0943106	0.1578684	20.01
Overall	0.946	0.824-1.087	-0.055513	-	100

Heterogeneity	Chi^2 (d.f.)	P	I^2 (%)
	0.09 (2)	0.956	0.0

Test of Overall Effect	z	P
	0.78	0.434

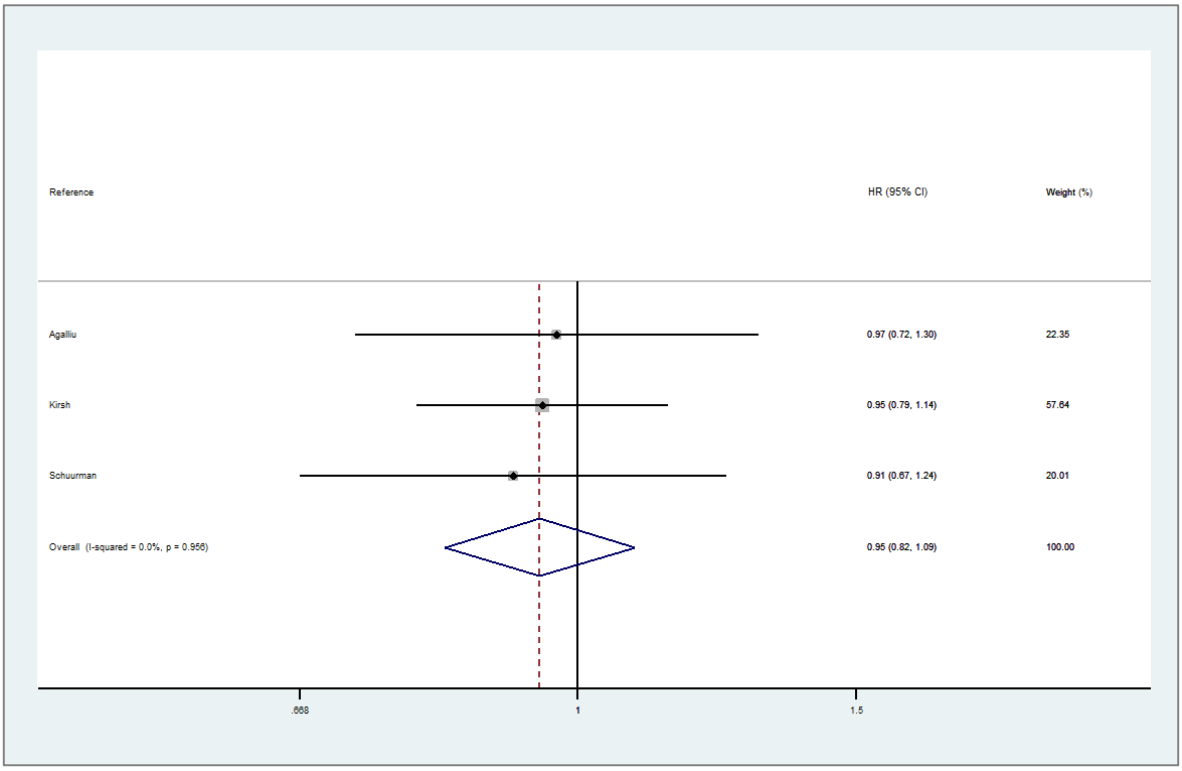

Figure Q - Forrest Plot Showing Risk Estimates from Included Cohort/Case-Cohort Studies of Dietary Lutein & Zeaxanthin Intake and Prostate Cancer Risk

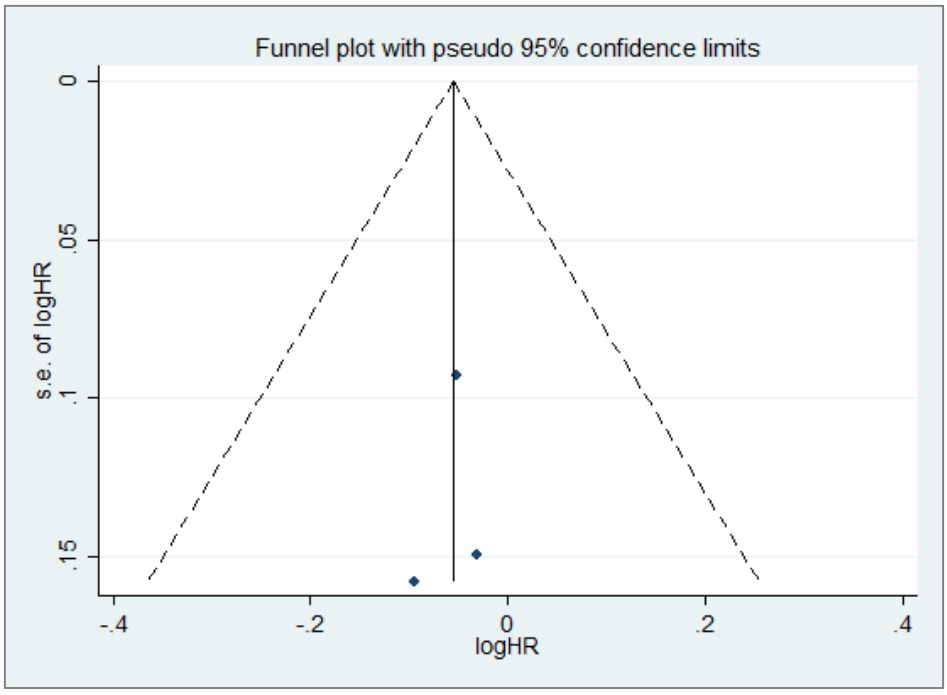

Figure R - Funnel Plot Examining Publication Bias in Included Cohort/Case-Cohort Studies of Dietary Lutein & Zeaxanthin and Prostate Cancer Risk

Appendix 7 – Meta-Analysis of Case-Control/Nested Case-Control Studies of Dietary Carotenoids and Prostate Cancer Risk Excluding Results from Jian et al

7.1. α-Carotene

Table I - Full Results of Included Studies

Reference	OR	95% CI	Log OR	SE	Weight (%)
Bosetti[56]	0.85	0.66-1.11	-0.1625189	0.1361627	10.11
Cohen[59]	0.75	0.51–1.09	-0.2876821	0.1907448	5.15
Deneo-Pellegrini[60]	0.9	0.5-1.6	-0.1053605	0.2935531	2.18
Hodge[57]	0.8	0.6-1.1	-0.2231435	0.1624764	7.10
Jain[61]	1.06	0.79-1.43	0.0582689	0.1527579	8.03
Lu[51]	0.47	0.14–1.66	-0.7550226	0.643796	0.45
McCann[55]	0.91	0.59-1.39	-0.0943106	0.2161298	4.01
Meyer[62]	1	0.53-1.89	0	0.3247841	1.78
Rohrmann[48]	0.96	0.87-1.07	-0.040822	0.0553473	61.19
Overall	0.926	0.851-1.008	-0.076881	-	100

Heterogeneity	Chi^2 (d.f.)	P	I^2 (%)
	4.82 (8)	0.777	0.0

Test of Overall Effect	Z	P
	1.78	0.076

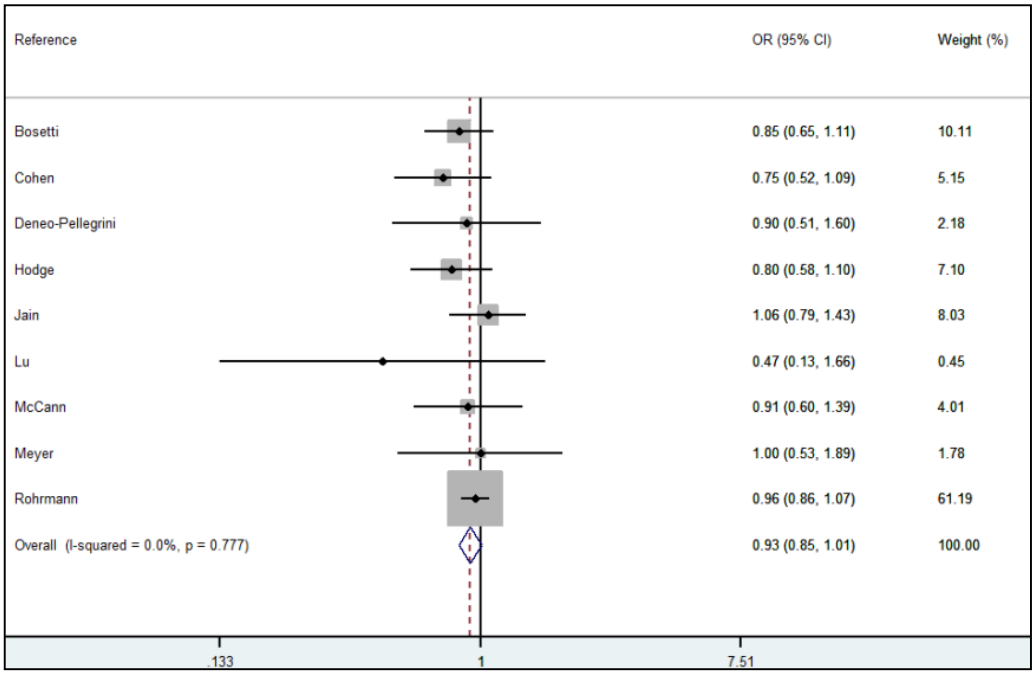

Figure S - Forrest Plot Showing Risk Estimates from Included Studies of Dietary α-Carotene Intake and Prostate Cancer Risk

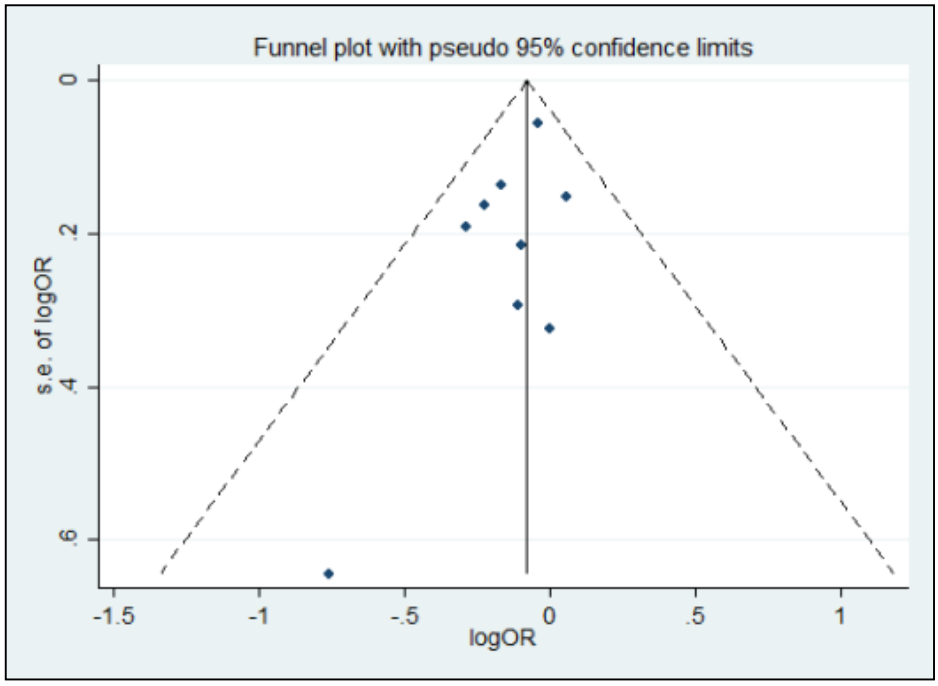

Figure T - Funnel Plot Examining Publication Bias in Included Studies of Dietary α-Carotene and Prostate Cancer Risk

7.2. β-Cryptoxanthin

Table J - Full Results of Included Studies

Reference	OR	95% CI	log OR	SE	Weight (%)
Bosetti[56]	0.9	0.69–1.16	-0.1053605	0.1294799	11.43
Cohen[59]	0.93	0.64-1.36	-0.0725707	0.1939058	5.10
Hodge[57]	0.9	0.7–1.3	-0.1053605	0.1876147	5.44
Jain[61]	1.44	1.09-1.89	0.3646432	0.1387417	9.95
Lu[51]	0.92	0.26-3.2	-0.0833816	0.6359859	0.47
McCann[55]	0.92	0.64-1.33	-0.0833816	0.1880411	5.42
Rohrmann[48]	0.87	0.79-0.97	-0.1392621	0.0555117	62.18
Overall	0.926	0.850-1.009	-0.076881	-	100

Heterogeneity	**Chi^2 (d.f.)**	**P**	**I^2 (%)**
	11.46 (6)	0.075	47.7
Test of Overall Effect	**z**	**P**	
	1.75	0.080	

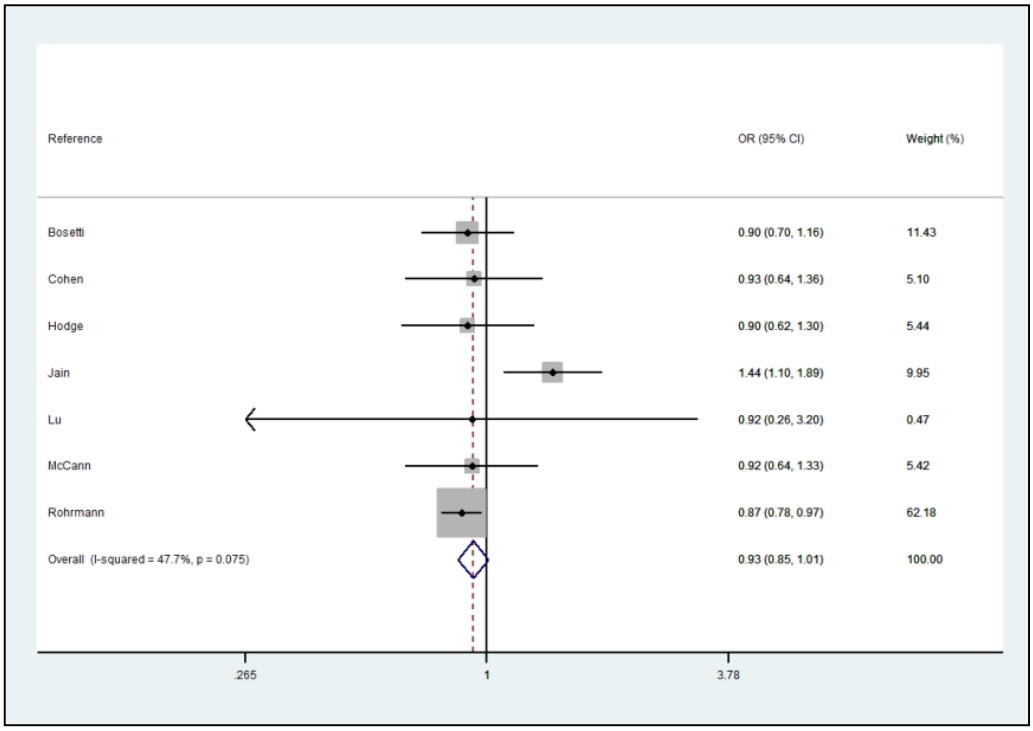

Figure U - Forrest Plot Showing Risk Estimates from Included Studies of Dietary β-Cryptoxanthin Intake and Prostate Cancer Risk

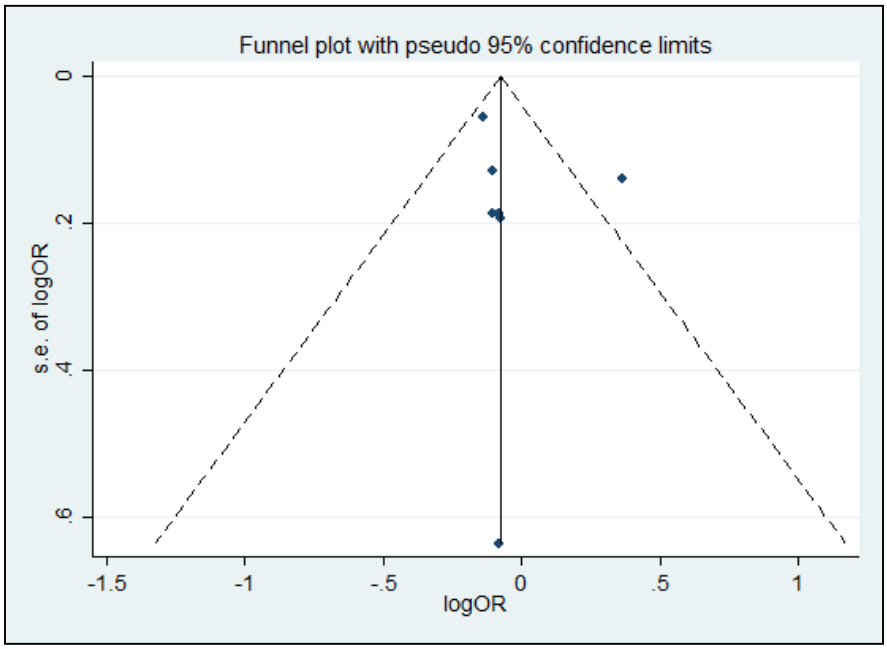

Figure V - Funnel Plot Examining Publication Bias in Included Studies of Dietary β-Cryptoxanthin and Prostate Cancer Risk

7.3. Lutein & Zeaxanthin

Table K - Full Results of Included Studies

Reference	OR	95% CI	log OR	SE	Weight (%)
Bosetti[56]	0.91	0.69-1.2	-0.0943106	0.1411389	9.16
Cohen[59]	0.68	0.45-1	-0.3856625	0.1967666	4.72
Hodge[57]	0.9	0.7-1.3	-0.1053605	0.1876147	5.19
Rohrmann[48]	0.82	0.74-0.9	-0.198451	0.0474951	80.93
Overall	0.825	0.758-0.897	-0.1923729	-	100

Heterogeneity	Chi^2 (d.f.)	P	I^2 (%)
	1.68 (3)	0.642	0.0

Test of Overall Effect	z	P
	4.51	0.000

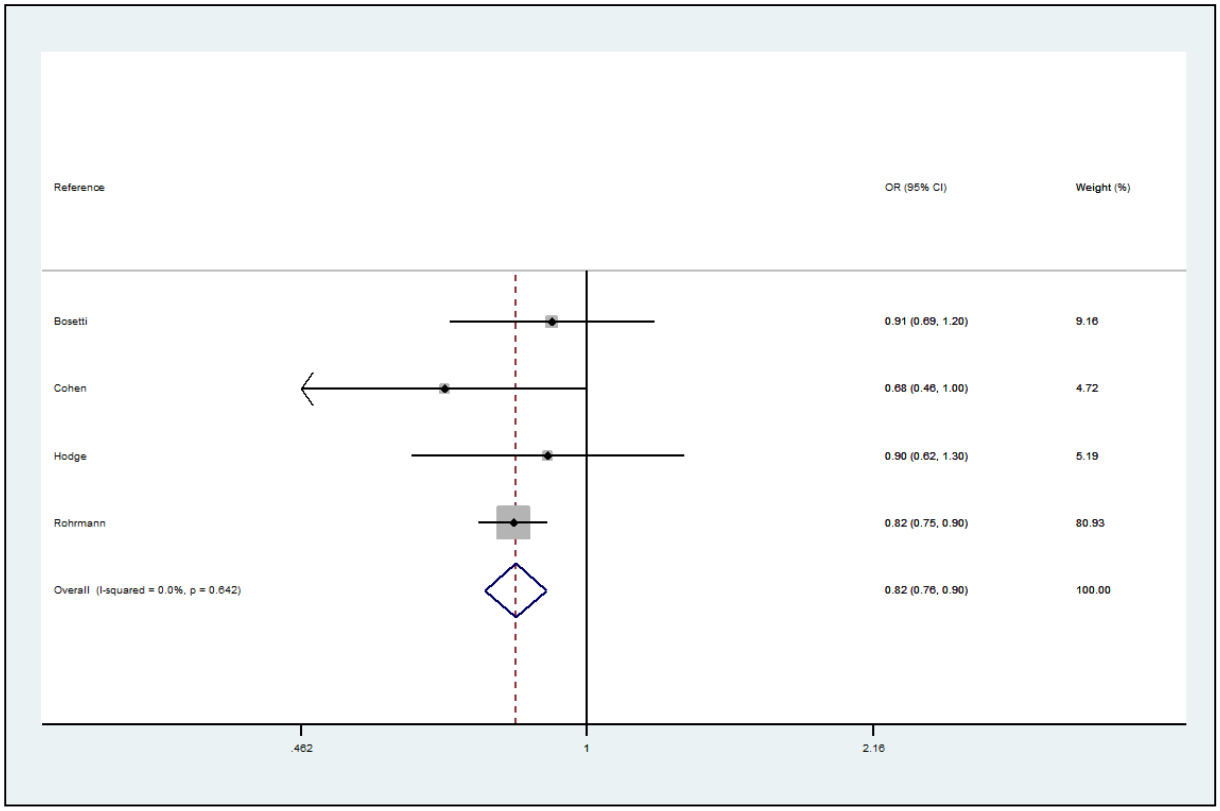

Figure W - Forrest Plot Showing Risk Estimates from Included Studies of Dietary lutein & Zeaxanthin Intake and Prostate Cancer Risk

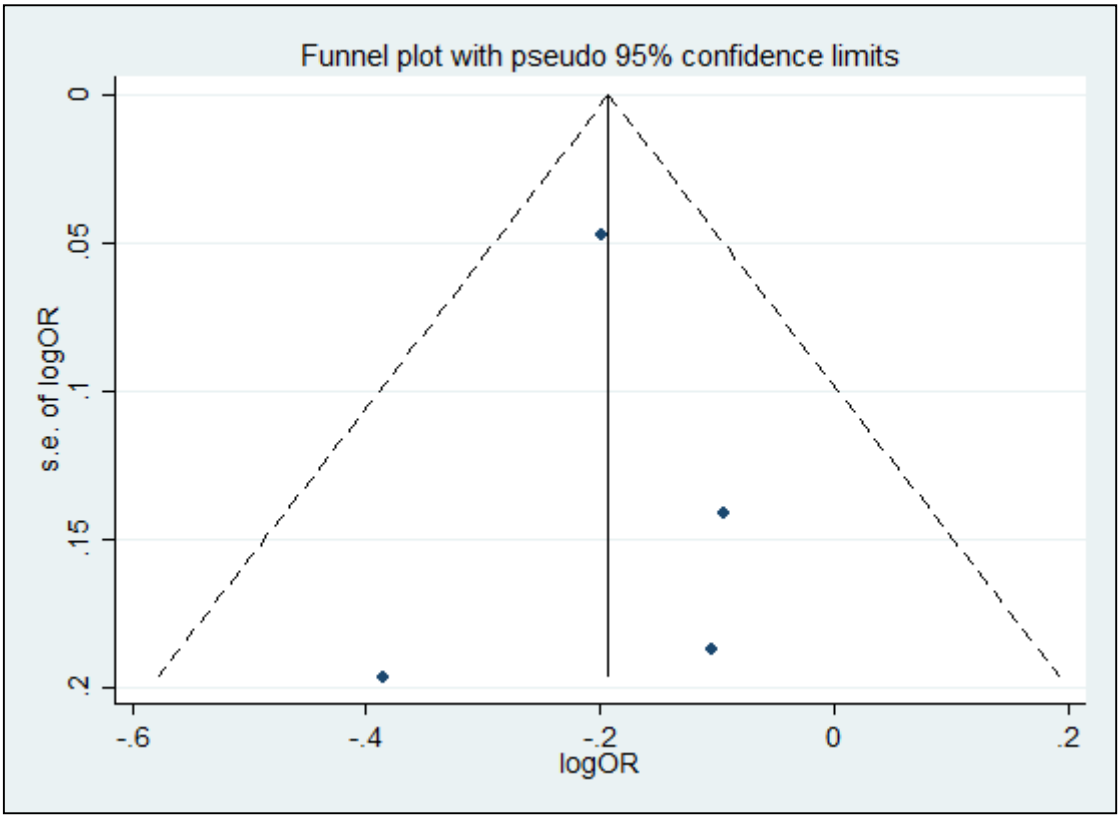

Figure X - Funnel Plot Examining Publication Bias in Included Studies of Dietary lutein & Zeaxanthin and Prostate Cancer Risk

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