Published in final edited form as:

Prostate. 2017 May ; 77(6): 647–653. doi:10.1002/pros.23312.

Circulating antioxidant levels and risk of prostate cancer by *TMPRSS2:ERG*

Rebecca E. Graff^{1,2}, Gregory Judson³, Thomas U. Ahearn¹, Michelangelo Fiorentino^{1,4,5}, Massimo Loda^{4,6}, Edward L. Giovannucci^{1,7,8}, Lorelei A. Mucci^{1,7}, and Andreas Pettersson^{1,9}

¹Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, MA, USA

²Department of Epidemiology & Biostatistics, University of California San Francisco, San Francisco, CA, USA

³Department of Medicine, University of California San Francisco, San Francisco, CA, USA

⁴Center for Molecular Oncologic Pathology, Dana-Farber Cancer Institute, Boston, MA, USA

⁵Pathology Unit, Addarii Institute, S. Orsola-Malpighi Hospital, Bologna, Italy

⁶Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA

⁷Channing Division of Network Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

⁸Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, MA, USA

⁹Clinical Epidemiology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden

Abstract

Background—Few studies have considered etiological differences across molecular subtypes of prostate cancer, despite potential to improve opportunities for precision prevention of a disease for which modifiable risk factors have remained elusive. Factors that lead to DNA double-strand breaks, such as oxidative stress, may promote the formation of the *TMPRSS2:ERG* gene fusion in prostate cancer. We tested the hypothesis that increasing levels of pre-diagnostic circulating antioxidants, which may reduce oxidative stress, are associated with lower risk of developing *TMPRSS2:ERG* positive prostate cancer.

Methods—We conducted a nested case-control study, including 370 cases and 2,470 controls, to evaluate associations between pre-diagnostic α - and β -carotene, α - and γ -tocopherol, β -cryptoxanthin, lutein, lycopene, retinol, and selenium with the risk of prostate cancer by *ERG* protein expression status (a marker of *TMPRSS2:ERG*). Multivariable unconditional polytomous logistic regression was used to calculate odds ratios and 95% confidence intervals.

Results—We did not find any of the antioxidants to be significantly associated with the risk of prostate cancer according to ERG status.

Conclusions—The results do not support the hypothesis that circulating pre-diagnostic antioxidant levels protect against developing *TMPRSS2:ERG* positive prostate cancer. Additional studies are needed to explore mechanisms for the development of *TMPRSS2:ERG* positive prostate cancer.

Keywords

biomarkers; molecular subtypes; epidemiology

INTRODUCTION

Gene fusions involving members of the ETS family of transcription factors define the largest molecular subgroup of primary prostate cancer [1]. *TMPRSS2:ERG* is the most common such gene fusion, occurring in 40-50% of primary tumors [2]. It is thus imperative to identify risk factors that could improve opportunities for precision prevention of *TMPRSS2:ERG* defined disease. Experimental evidence suggests that androgen exposure together with factors that lead to DNA double-strand breaks, such as radiation, promotes the formation of *TMPRSS2:ERG* [3-5]. Oxidative stress can induce double-strand breaks, lending plausibility to the hypothesis that increasing levels of circulating antioxidants, which may reduce oxidative stress, could be associated with a lower risk of developing *TMPRSS2:ERG* positive prostate cancer. Were the hypothesis true, then it could explain some of the mixed evidence regarding associations between antioxidants and prostate cancer overall [6]. I.e., if studies of antioxidants and prostate cancer have been conducted in populations with different prevalences of molecular subtypes, then one would expect to have found varying strengths of association. To evaluate the hypothesis, we conducted a nested case-control study of 370 prostate cancer cases and 2,470 controls assessing the associations between pre-diagnostic circulating levels of α - and β -carotene, α - and γ -tocopherol, β -cryptoxanthin, lutein, lycopene, and retinol and risk of prostate cancer by ERG protein expression status (a marker of *TMPRSS2:ERG* fusion status).

MATERIALS AND METHODS

Study population

This study included prostate cancer cases with known ERG protein expression status and controls from several previous studies of pre-diagnostic circulating antioxidant levels nested within the Physicians' Health Study (PHS) and Health Professionals Follow-up Study (HPFS) cohorts [7-11]. The PHS was a randomized, double-blind, placebo-controlled trial of aspirin and β -carotene supplementation initiated in 1982 among 22,071 male physicians ages 40 to 84 [12]. The HPFS is an ongoing prospective cohort study of 51,529 male health professionals ages 40 to 75 at enrollment in 1986. Additional details of the cohorts can be found in the previously conducted studies [7-11].

In the PHS, blood was collected from 68% of participants (n = 14,916) prior to randomization, as previously described [13]. From among those who provided blood,

incident prostate cancer cases (diagnosed through 2005) and controls were selected via risk-set sampling and additionally matched on age at baseline and smoking status (never, former, current). In the HPFS, blood was collected from 18,159 participants (35% of the cohort) free from prostate cancer between 1993 and 1995, as previously described [11]. From among those who provided blood, incident prostate cancer cases and controls who had had a prostate-specific antigen (PSA) test after blood draw were selected using risk-set sampling at three time points (1996, 1998, and 2000). Cases and controls were additionally matched on year of birth, PSA test before blood draw (yes, no), timing of blood draw (midnight – before 9 am, 9 am – before noon, noon – 4 pm, and after 4 pm – before midnight), season of draw (winter, spring, summer, fall), and year of draw (exact).

In total, antioxidant data for at least one biomarker of interest were available for 1,749 cases and 2,511 controls. Of the cases, ERG tumor status was available for 375 (PHS: 209 / HPFS: 166). After excluding 3 cases with T1a tumors, 2 cases and 22 controls who had a diagnosis of cancer other than nonmelanoma skin cancer before the date of blood draw, 5 controls with missing index dates for matching, and 14 controls with a blood draw date after the date of their matched case's diagnosis, 370 unique cases and 2,470 unique controls remained for analyses. Among them, 11 individuals served as both a control prior to prostate cancer diagnosis and as a case.

This study was approved by the Human Subjects Committee at the Harvard T.H. Chan School of Public Health and by Partners Health Care. Written informed consent was obtained from each subject.

Measurement of antioxidant levels

The measurement of circulating antioxidants levels has been previously described in detail [7-11]. In brief, in the PHS, plasma was assayed for α -carotene, α - and γ -tocopherol, β -cryptoxanthin, lutein, lycopene, and retinol in three batches [7,8]. β -carotene and selenium were assayed in two batches [9]. In the HPFS, α - and β -carotene, α - and γ -tocopherol, β -cryptoxanthin, lutein, and lycopene were assayed in three batches [10,11], and retinol and selenium were not measured. For samples from both the PHS and the HPFS, cases and their matched controls were analyzed together and laboratory personnel were unable to distinguish case, control and quality control samples.

Tumor tissue collection and assessment of *TMPRSS2:ERG* status

In both the PHS and HPFS, prostate tumor tissue has been collected from participants having undergone radical prostatectomy or transurethral resection of the prostate (TURP). Tissue microarrays have been constructed by taking three or more 0.6-mm cores of tissue from the primary tumor nodule or nodule with the highest Gleason grade.

We characterized presence or absence of *TMPRSS2:ERG* by immunohistochemical assessment of ERG protein expression as previously described [14]. Several studies have shown ERG protein expression to be strongly correlated with *TMPRSS2:ERG* fusion status as assessed by other methods [15-17].

Statistical analysis

We calculated batch-specific quartiles of antioxidants levels according to the distribution in the controls. We then used unconditional binary logistic regression to estimate odds ratios (OR) and 95% confidence intervals (CI) for associations between plasma antioxidants and risk of prostate cancer overall, and unconditional polytomous logistic regression to study associations with three outcomes: ERG-positive prostate cancer, ERG-negative prostate cancer, and controls. To maximize power, we combined the two cohorts and adjusted for all matching factors used in either cohort. We also ran models additionally adjusted for body mass index (BMI) at blood draw and cohort. Missing data for covariates (<10% for all covariates) were assigned to the mode value for categorical variables and the median value for continuous variables. We conducted tests for linear trend by modeling quartile scores as continuous terms (0, 1, 2, or 3) and calculating the Wald statistic. To test for heterogeneity by ERG status, we evaluated the Wald statistic from comparing the trend parameters for ERG-positive disease versus control and for ERG-negative versus control. We ran sensitivity analyses restricted to Caucasian men, excluding 29 cases with ERG assayed in TURP specimens or tissue from an unknown source, excluding cases diagnosed within one year of blood draw, by cohort separately, and using conditional logistic regression of matched sets. Analyses were conducted using SAS version 9.2 (SAS Institute, Inc. Cary, NC). All tests were two-sided and *p*-values below 0.05 were considered statistically significant.

RESULTS

Characteristics of the 2,470 controls, 191 ERG-negative cases and 179 ERG-positive cases are presented in **Table 1**. The mean time between blood draw and diagnosis for all cases was 7.6 years. Cases were more likely than controls to have ever had a PSA test prior to blood draw, and to have had their blood drawn in winter or summer; controls were more likely to have had their blood drawn in fall. Among cases only, as previously reported [14], those who were ERG-positive were younger at diagnosis and had higher stage tumors relative to those who were ERG-negative. Batch-specific median antioxidant levels in both cases and controls are available in **Supplementary Table S1**. The cases excluded from our analyses because they lacked antioxidant and/or ERG expression data were more likely missing data regarding the clinical characteristics of their disease. In addition, their distribution of clinical stage was shifted slightly upwards, they were diagnosed in later years at an older age with higher PSA levels, and they were less likely to be treated with radical prostatectomy (data not shown).

Table 2 presents associations between quartiles of circulating antioxidants and risk of prostate cancer overall and by ERG status. None of the biomarkers were significantly associated with prostate cancer overall. Lutein, however, demonstrated a nonsignificant inverse relationship (top vs. bottom quartile OR: 0.73; 95% CI: 0.52-1.01; P_{trend} : 0.08) and selenium showed suggestion of a positive association (OR: 1.57; 95% CI: 0.92-2.69; P_{trend} : 0.09).

Polytomous models assessing the risk of ERG-negative and ERG-positive cancer separately did not suggest differential associations between any of the circulating antioxidants and risk of prostate cancer according to ERG status (**Table 2**). α -tocopherol was non-significantly associated with a decreased risk of ERG-positive disease (OR_{Q4 vs. Q1}: 0.76; 95% CI:

0.48-1.20; P_{trend} : 0.08), but not with the risk of ERG-negative disease ($\text{OR}_{\text{Q4 vs. Q1}}$: 1.03; 95% CI: 0.66-1.61; P_{trend} : 0.82) (P_{diff} : 0.13). All other P_{diff} for differential associations according to ERG status were greater than 0.30. Results from all sensitivity analyses were materially unchanged.

DISCUSSION

In this first study to examine the relationship between circulating pre-diagnostic antioxidant levels and *TMPRSS2:ERG* defined prostate cancer, we did not find any significant associations. Our results do not support the hypothesis that higher circulating pre-diagnostic antioxidant levels protect against developing *TMPRSS2:ERG* positive prostate cancer specifically.

Our results are somewhat surprising in the context of two recent studies suggesting a relationship between exposures that reduce genotoxic stress and a lower risk of prostate cancer harboring *TMPRSS2:ERG* [18,19]. In a study from our group, we found that the intake of tomato sauce, a substantial contributor to lycopene intake, is more strongly associated with ERG-positive than ERG-negative prostate cancer [18]. Similarly, Wright and colleagues determined that the use of aspirin, an anti-inflammatory medication [20], is associated with a significantly reduced risk of *TMPRSS2:ERG* positive prostate cancer only [19]. It could be that tomato sauce and aspirin have properties other than antioxidants that contribute to a reduced risk of *TMPRSS2:ERG* positive disease. It is also possible that the specific circulating antioxidant exposures in the current study were not relevant to the risk of prostate cancer. In particular, antioxidant exposure at a different time point could be important in the development of *TMPRSS2:ERG*; antioxidants were measured an average of approximately seven to eight years before diagnosis in our study. Our results could also be explained by chance.

Although our results for pre-diagnostic circulating antioxidant levels were largely null, it remains possible that oxidative stress and antioxidants could play a role in the development of *TMPRSS2:ERG* positive prostate cancer. For example, androgen exposure can increase oxidative stress in prostate cancer cell lines [21]. It could be that the local increase in oxidative stress precipitated by increased androgen signaling outweighs any potentially beneficial effects of high circulating antioxidant levels for *TMPRSS2:ERG* positive disease. Moreover, the tissue microenvironment in which tumors develop is a critical factor in cancer initiation and progression [22]. Our lack of association in this study could be explained by our lack of data regarding the tumor microenvironment.

An additional possibility is that the *TMPRSS2:ERG* gene fusion may not directly result from oxidative stress. Androgen signaling has previously been associated with co-recruitment of androgen receptor and topoisomerase II beta (TOP2B) and with TOP2B-mediated DNA double-strand breaks at sites of the fusion [23]. It is possible that these strand breaks could lead to the formation of *TMPRSS2:ERG* in a manner independent of strand breaks induced by oxidative stress. It also possible, however, that TOP2B is recruited to the site of *TMPRSS2:ERG* fusions in response to strand breaks resulting from genotoxic insults.

We did observe some indication that increasing α -tocopherol levels could be exclusively associated with ERG-positive prostate cancer. Again, the suggestive results could be due to chance alone. *A priori*, we did not anticipate that α -tocopherol would stand out from the other antioxidants evaluated in this study. However, a recent pooled analysis of circulating carotenoids, retinol, and tocopherols found only α -tocopherol to be associated with a significantly reduced risk of prostate cancer [24]. It could be that the foods that contribute to circulating α -tocopherol levels have other components that affect prostate cancer risk, and specifically the risk of *TMPRSS2:ERG* positive prostate cancer.

This study was limited by the relatively small number of cases assayed for ERG status. We also measured ERG protein expression rather than the *TMPRSS2:ERG* fusion itself, but the former has been shown to have high concordance with the latter as assessed by alternative methods [15-17]. It is also important to note that cases assayed for ERG status are largely those treated with radical prostatectomy. As a result, they may not be representative of all men diagnosed with prostate cancer. Still, our study was borne from a well-annotated prospective cohort with tumor tissue, plasma, and clinical data. We were able to measure circulating antioxidant levels prior to diagnosis, thereby sidestepping any issues of reverse causation, and we adjusted for potentially important confounding variables.

CONCLUSIONS

In summary, we did not find credible evidence that pre-diagnostic circulating antioxidant levels are associated with the risk of *TMPRSS2:ERG* defined prostate cancer. Additional studies are needed to explore mechanisms for the development of disease positive for the fusion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We would like to thank the participants and staff of the PHS and HPFS for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data. We would like to acknowledge Dr. Howard D. Sesso for his valuable contributions to the PHS and Dr. Walter C. Willett for his valuable contributions to the HPFS. The TMAs were constructed by the Tissue Microarray Core Facility at the Dana Farber/Harvard Cancer Center.

Funding: This work was supported by the Dana-Farber / Harvard Cancer Center Specialized Programs of Research Excellence (SPORE) in Prostate Cancer (P50 CA090381); the National Cancer Institute at the National Institutes of Health (R25 CA112355 to R.E.G., T32 CA09001 to T.U.A., R01 CA136578, UM1CA167552); and the American Cancer Society – Ellison Foundation Postdoctoral Fellowship (PF-14-140-01-CCE to T.U.A.). L.A.M. is a Prostate Cancer Foundation Young Investigator.

REFERENCES

1. The Cancer Genome Atlas Research Network. The molecular taxonomy of primary prostate cancer. *Cell*. 2015; 163(4):1011–1025. [PubMed: 26544944]
2. Tomlins SA, Laxman B, Dhanasekaran SM, Helgeson BE, Cao X, Morris DS, Menon A, Jing X, Cao Q, Han B, Yu J, Wang L, Montie JE, Rubin MA, Pienta KJ, Roulston D, Shah RB, Varambally

- S, Mehra R, Chinnaiyan AM. Distinct classes of chromosomal rearrangements create oncogenic ETS gene fusions in prostate cancer. *Nature*. 2007; 448(7153):595–599. [PubMed: 17671502]
3. Mani RS, Chinnaiyan AM. Triggers for genomic rearrangements: insights into genomic, cellular and environmental influences. *Nat Rev Genet*. 2010; 11(12):819–829. [PubMed: 21045868]
 4. Lin C, Yang L, Tanasa B, Hutt K, Ju BG, Ohgi K, Zhang J, Rose DW, Fu XD, Glass CK, Rosenfeld MG. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. *Cell*. 2009; 139(6):1069–1083. [PubMed: 19962179]
 5. Mani RS, Amin MA, Li X, Kalyana-Sundaram S, Veeneman BA, Wang L, Ghosh A, Aslam A, Ramanand SG, Rabquer BJ, Kimura W, Tran M, Cao X, Roychowdhury S, Dhanasekaran SM, Palanisamy N, Sadek HA, Kapur P, Koch AE, Chinnaiyan AM. Inflammation-Induced Oxidative Stress Mediates Gene Fusion Formation in Prostate Cancer. *Cell Rep*. 2016; 17(10):2620–2631. [PubMed: 27926866]
 6. Chan JM, Gann PH, Giovannucci EL. Role of diet in prostate cancer development and progression. *J Clin Oncol*. 2005; 23(32):8152–8160. [PubMed: 16278466]
 7. Cook NR, Stampfer MJ, Ma J, Manson JE, Sacks FM, Buring JE, Hennekens CH. Beta-carotene supplementation for patients with low baseline levels and decreased risks of total and prostate carcinoma. *Cancer*. 1999; 86(9):1783–1792. [PubMed: 10547552]
 8. Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH, Stampfer MJ. Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. *Cancer Res*. 1999; 59(6):1225–1230. [PubMed: 10096552]
 9. Li H, Stampfer MJ, Giovannucci EL, Morris JS, Willett WC, Gaziano JM, Ma J. A prospective study of plasma selenium levels and prostate cancer risk. *J Natl Cancer Inst*. 2004; 96(9):696–703. [PubMed: 15126606]
 10. Van Blarigan EL, Ma J, Kenfield SA, Stampfer MJ, Sesso HD, Giovannucci EL, Witte JS, Erdman JW Jr, Chan JM, Penney KL. Plasma antioxidants, genetic variation in SOD2, CAT, GPX1, GPX4, and prostate cancer survival. *Cancer Epidemiol Biomarkers Prev*. 2014; 23(6):1037–1046. [PubMed: 24711484]
 11. Wu K, Erdman JW Jr, Schwartz SJ, Platz EA, Leitzmann M, Clinton SK, DeGroot V, Willett WC, Giovannucci E. Plasma and dietary carotenoids, and the risk of prostate cancer: a nested case-control study. *Cancer Epidemiol Biomarkers Prev*. 2004; 13(2):260–269. [PubMed: 14973107]
 12. Hennekens CH, Eberlein K. A randomized trial of aspirin and beta-carotene among U.S. physicians. *Prev Med*. 1985; 14(2):165–168. [PubMed: 3900975]
 13. Gann PH, Hennekens CH, Ma J, Longcope C, Stampfer MJ. Prospective study of sex hormone levels and risk of prostate cancer. *J Natl Cancer Inst*. 1996; 88(16):1118–1126. [PubMed: 8757191]
 14. Pettersson A, Graff RE, Bauer SR, Pitt MJ, Lis RT, Stack EC, Martin NE, Kunz L, Penney KL, Ligon AH, Suppan C, Flavin R, Sesso HD, Rider JR, Sweeney C, Stampfer MJ, Fiorentino M, Kantoff PW, Sanda MG, Giovannucci EL, Ding EL, Loda M, Mucci LA. The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2012; 21(9):1497–1509. [PubMed: 22736790]
 15. Chaux A, Albaldine R, Toubaji A, Hicks J, Meeker A, Platz EA, De Marzo AM, Netto GJ. Immunohistochemistry for ERG expression as a surrogate for TMPRSS2-ERG fusion detection in prostatic adenocarcinomas. *Am J Surg Pathol*. 2011; 35(7):1014–1020. [PubMed: 21677539]
 16. Park K, Tomlins SA, Mudaliar KM, Chiu YL, Esgueva R, Mehra R, Suleman K, Varambally S, Brenner JC, MacDonald T, Srivastava A, Tewari AK, Sathyanarayana U, Nagy D, Pestano G, Kunju LP, Demichelis F, Chinnaiyan AM, Rubin MA. Antibody-based detection of ERG rearrangement-positive prostate cancer. *Neoplasia*. 2010; 12(7):590–598. [PubMed: 20651988]
 17. van Leenders GJ, Boormans JL, Vissers CJ, Hoogland AM, Bressers AA, Furusato B, Trapman J. Antibody EPR3864 is specific for ERG genomic fusions in prostate cancer: implications for pathological practice. *Mod Pathol*. 2011; 24(8):1128–1138. [PubMed: 21499236]
 18. Graff RE, Pettersson A, Lis RT, Ahearn TU, Markt SC, Wilson KM, Rider JR, Fiorentino M, Finn S, Kenfield SA, Loda M, Giovannucci EL, Rosner B, Mucci LA. Dietary lycopene intake and risk of prostate cancer defined by ERG protein expression. *Am J Clin Nutr*. 2016; 103(3):851–860. [PubMed: 26817504]

19. Wright JL, Chery L, Holt S, Lin DW, Luedeke M, Rinckleb AE, Maier C, Stanford JL. Aspirin and NSAID use in association with molecular subtypes of prostate cancer defined by TMPRSS2:ERG fusion status. *Prostate Cancer Prostatic Dis.* 2016; 19(1):53–56. [PubMed: 26503111]
20. Vane J. The evolution of non-steroidal anti-inflammatory drugs and their mechanisms of action. *Drugs.* 1987; 33(Suppl 1):18–27. [PubMed: 3595474]
21. Ripple MO, Henry WF, Rago RP, Wilding G. Prooxidant-antioxidant shift induced by androgen treatment of human prostate carcinoma cells. *J Natl Cancer Inst.* 1997; 89(1):40–48. [PubMed: 8978405]
22. Quail DF, Joyce JA. Microenvironmental regulation of tumor progression and metastasis. *Nat Med.* 2013; 19(11):1423–1437. [PubMed: 24202395]
23. Haffner MC, Aryee MJ, Toubaji A, Esopi DM, Albadine R, Gurel B, Isaacs WB, Bova GS, Liu W, Xu J, Meeker AK, Netto G, De Marzo AM, Nelson WG, Yegnasubramanian S. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. *Nat Genet.* 2010; 42(8):668–675. [PubMed: 20601956]
24. Key TJ, Appleby PN, Travis RC, Albanes D, Alberg AJ, Barricarte A, Black A, Boeing H, Bueno-de-Mesquita HB, Chan JM, Chen C, Cook MB, Donovan JL, Galan P, Gilbert R, Giles GG, Giovannucci E, Goodman GE, Goodman PJ, Gunter MJ, Hamdy FC, Heliovaara M, Helzlsouer KJ, Henderson BE, Herberg S, Hoffman-Bolton J, Hoover RN, Johansson M, Khaw KT, King IB, Knekt P, Kolonel LN, Le Marchand L, Mannisto S, Martin RM, Meyer HE, Mondul AM, Moy KA, Neal DE, Neuhauser ML, Palli D, Platz EA, Pouchieu C, Rissanen H, Schenk JM, Severi G, Stampfer MJ, Tjonneland A, Touvier M, Trichopoulou A, Weinstein SJ, Ziegler RG, Zhou CK, Allen NE. Carotenoids, retinol, tocopherols, and prostate cancer risk: pooled analysis of 15 studies. *Am J Clin Nutr.* 2015; 102(5):1142–1157. [PubMed: 26447150]

Table 1

Characteristics of prostate cancer cases by ERG status and matched controls in the Physicians' Health Study and Health Professionals Follow-up Study

	Controls	ERG- Cases	ERG+ Cases	<i>P</i> -diff ^a
Number	2470	191	179	
Caucasian, n (%)	2328 (96.0%)	181 (96.3%)	168 (96.6%)	0.89
Characteristics at Blood Draw				
Mean Age, years (SD)	61.2 (8.4)	58.4 (8.6)	57.0 (8.0)	0.10
Mean BMI, kg/m ² (SD)	25.0 (2.9)	25.0 (2.8)	24.7 (2.6)	0.29
Current Smoker, n (%)	200 (8.1%)	15 (7.9%)	15 (8.4%)	0.85
Ever PSA Test at Time of Blood Draw, n (%)	464 (19.1%)	54 (28.9%)	58 (32.4%)	0.46
Time of Blood Draw, n (%)				
Midnight - Before 9am	650 (28.6%)	47 (26.7%)	45 (27.3%)	
9am - Before 12pm	1155 (50.8%)	93 (52.8%)	78 (47.3%)	
12pm - Before 4pm	423 (18.6%)	33 (18.8%)	38 (23.0%)	
4pm - Before Midnight	47 (2.1%)	3 (1.7%)	4 (2.4%)	0.69
Season of Blood Draw, n (%)				
Winter	368 (14.9%)	40 (20.9%)	34 (19.0%)	
Spring	358 (14.5%)	28 (14.7%)	26 (14.5%)	
Summer	279 (11.3%)	30 (15.7%)	30 (16.8%)	
Fall	1465 (59.3%)	93 (48.7%)	89 (49.7%)	0.97
Characteristics at Diagnosis				
Mean Time from Blood Draw to Diagnosis, years (SD)	-	7.7 (5.4)	7.4 (4.9)	0.50
Mean Age, years (SD)	-	66.2 (6.6)	64.5 (6.5)	0.009
Mean PSA, ng/mL (SD)	-	14.0 (40.2)	8.4 (6.8)	0.08
Pathological Stage, n (%) ^b				
T2 N0/NX	-	119 (76.3%)	107 (64.1%)	
T3 N0/NX	-	37 (23.7%)	51 (30.5%)	
T4/N1/M1	-	0 (0.0%)	9 (5.4%)	0.003
Gleason Grade, n (%)				
2-6	-	43 (24.3%)	30 (17.1%)	
3+4	-	55 (31.1%)	76 (43.2%)	
4+3	-	46 (26.0%)	37 (21.0%)	
8-10	-	33 (18.6%)	33 (18.8%)	0.82
Year of Diagnosis, n (%)				
1983-1990	-	26 (13.6%)	22 (12.3%)	
1991-1995	-	63 (33.0%)	72 (40.2%)	
1996-2000	-	102 (53.4%)	85 (47.5%)	0.53

NOTE: Numbers may not add up as expected for characteristics with missing data; percentages may not add up as expected due to rounding

^a *P*-value for difference between ERG- and ERG+; based on χ^2 tests for race, smoking status at blood draw, ever PSA test at blood draw, season of blood draw; Fisher's exact test for time of blood draw, *t*-tests for age at blood draw and diagnosis, BMI at blood draw, time from blood draw to diagnosis, PSA at diagnosis; Cochran-Armitage trend test for pathological stage (exact), Gleason grade, year of diagnosis

^bRestricted to 323 radical prostatectomy specimens with stage data available (out of 341 radical prostatectomy specimens total)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2

Odds ratios and 95% confidence intervals for batch-specific quartiles of plasma antioxidant levels and risk of ERG-negative and, separately, ERG-positive prostate cancer

	# Controls / ERG- / ERG+	All Case / Control OR (95% CI) ^a	ERG- / Control OR (95% CI) ^b	ERG+ / Control OR (95% CI) ^b	<i>p</i> -diff ^c
α-carotene					
Quartile 1	592 / 53 / 44	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Quartile 2	595 / 40 / 31	0.73 (0.52, 1.02)	0.75 (0.49, 1.16)	0.71 (0.44, 1.15)	
Quartile 3	596 / 53 / 56	1.16 (0.86, 1.58)	1.03 (0.68, 1.54)	1.34 (0.87, 2.04)	
Quartile 4	594 / 41 / 40	0.89 (0.64, 1.24)	0.81 (0.53, 1.26)	0.99 (0.62, 1.56)	0.35
<i>p</i> -trend ^d		0.84	0.65	0.43	
α-tocopherol					
Quartile 1	591 / 44 / 50	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Quartile 2	594 / 47 / 54	1.13 (0.83, 1.54)	1.11 (0.72, 1.71)	1.15 (0.76, 1.74)	
Quartile 3	596 / 50 / 34	0.94 (0.68, 1.30)	1.18 (0.77, 1.81)	0.72 (0.46, 1.14)	
Quartile 4	592 / 44 / 34	0.89 (0.64, 1.24)	1.03 (0.66, 1.61)	0.76 (0.48, 1.20)	0.13
<i>p</i> -trend ^d		0.32	0.82	0.08	
β-carotene					
Quartile 1	503 / 32 / 39	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Quartile 2	506 / 40 / 32	0.98 (0.69, 1.41)	1.20 (0.74, 1.96)	0.80 (0.49, 1.31)	
Quartile 3	506 / 30 / 40	0.99 (0.69, 1.43)	0.93 (0.55, 1.57)	1.05 (0.65, 1.69)	
Quartile 4	503 / 30 / 25	0.80 (0.54, 1.17)	0.94 (0.56, 1.59)	0.67 (0.39, 1.15)	0.73
<i>p</i> -trend ^d		0.29	0.59	0.31	
β-cryptoxanthin					
Quartile 1	588 / 44 / 44	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Quartile 2	591 / 48 / 37	0.93 (0.67, 1.30)	1.07 (0.70, 1.65)	0.79 (0.50, 1.26)	
Quartile 3	591 / 42 / 44	0.98 (0.71, 1.36)	0.95 (0.61, 1.49)	1.02 (0.65, 1.59)	
Quartile 4	590 / 54 / 45	1.17 (0.85, 1.61)	1.26 (0.82, 1.92)	1.08 (0.69, 1.68)	0.89
<i>p</i> -trend ^d		0.31	0.39	0.53	
γ-tocopherol					
Quartile 1	591 / 38 / 44	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Quartile 2	593 / 49 / 47	1.08 (0.78, 1.49)	1.21 (0.78, 1.89)	0.96 (0.62, 1.48)	
Quartile 3	594 / 51 / 31	0.98 (0.70, 1.37)	1.32 (0.85, 2.05)	0.69 (0.42, 1.11)	
Quartile 4	593 / 47 / 50	1.20 (0.87, 1.66)	1.25 (0.80, 1.96)	1.16 (0.75, 1.78)	0.58
<i>p</i> -trend ^d		0.38	0.30	0.80	
Lutein					
Quartile 1	590 / 50 / 48	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Quartile 2	594 / 52 / 46	0.96 (0.70, 1.31)	1.00 (0.66, 1.50)	0.92 (0.60, 1.41)	
Quartile 3	594 / 43 / 47	0.96 (0.70, 1.32)	0.89 (0.58, 1.37)	1.04 (0.68, 1.59)	
Quartile 4	592 / 43 / 30	0.73 (0.52, 1.01)	0.82 (0.53, 1.27)	0.62 (0.38, 1.01)	0.63

	# Controls / ERG- / ERG+	All Case / Control OR (95% CI) ^a	ERG- / Control OR (95% CI) ^b	ERG+ / Control OR (95% CI) ^b	<i>p</i> -diff ^c
<i>p</i> -trend ^d		0.08	0.31	0.11	
Lycopene					
Quartile 1	594 / 44 / 37	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Quartile 2	598 / 50 / 42	1.05 (0.76, 1.46)	1.05 (0.68, 1.61)	1.00 (0.63, 1.59)	
Quartile 3	598 / 44 / 53	1.08 (0.78, 1.49)	0.89 (0.57, 1.38)	1.21 (0.78, 1.89)	
Quartile 4	595 / 50 / 40	0.98 (0.70, 1.36)	1.00 (0.65, 1.53)	0.86 (0.54, 1.39)	0.95
<i>p</i> -trend ^d		0.91	0.80	0.75	
Retinol					
Quartile 1	426 / 22 / 24	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Quartile 2	428 / 21 / 22	0.97 (0.62, 1.51)	1.00 (0.54, 1.86)	0.93 (0.51, 1.71)	
Quartile 3	429 / 33 / 21	1.18 (0.77, 1.82)	1.49 (0.85, 2.63)	0.90 (0.49, 1.66)	
Quartile 4	427 / 29 / 25	1.03 (0.67, 1.57)	1.16 (0.65, 2.07)	0.92 (0.51, 1.66)	0.40
<i>p</i> -trend ^d		0.71	0.39	0.77	
Selenium					
Quartile 1	202 / 12 / 14	1.00 (ref)	1.00 (ref)	1.00 (ref)	
Quartile 2	204 / 21 / 18	1.49 (0.87, 2.56)	1.72 (0.82, 3.59)	1.28 (0.61, 2.68)	
Quartile 3	204 / 25 / 21	1.75 (1.03, 2.97)	2.03 (0.99, 4.17)	1.50 (0.73, 3.09)	
Quartile 4	202 / 20 / 23	1.57 (0.92, 2.69)	1.56 (0.74, 3.31)	1.59 (0.78, 3.25)	0.87
<i>p</i> -trend ^d		0.09	0.24	0.18	

^aFrom binary logistic regression models adjusted for age at blood draw (continuous), smoking status at blood draw (yes, no), ever PSA test prior to blood draw (yes, no), time of blood draw (midnight - 9am, 9am - before 12pm, 12pm - before 4pm, 4pm - before midnight), season of blood draw (winter, spring, summer, fall) and time between blood draw and index date (continuous)

^bFrom polytomous logistic regression models adjusted for the variables listed above

^cBased on the Wald chi-square statistic from comparing the exposure parameters for trend for each outcome versus control from the polytomous logistic regression model

^dCalculated by modeling the quartile ordinal score (0, 1, 2, 3) as a continuous variable