Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Dina Huang Date

Oxidative Stress, Gamma-tocopherol and Colorectal Adenoma
By

Dina Huang
Degree to be awarded: MPH

Epidemiology

_____

Michael Goodman
Committee Chair

_____

Committee Member

_____

Committee Member

_____

Committee Member

Oxidative Stress, Gamma-tocopherol and Colorectal Adenoma

By

Dina Huang

Bachelor of Science
Huazhong Agricultural University
2013

Thesis Committee Chair: Michael Goodman, MD MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2015

Abstract

Oxidative Stress, Gamma-tocopherol and Colorectal Adenoma
By Dina Huang

Background:
Oxidative stress is considered both the initiator and promoter of colorectal carcinogenesis. In this study we examined the use of gamma-tocopherol, a major isoform of vitamin E, as an oxidative biomarker and assessed its association with incident, sporadic colorectal adenoma. We conducted these analyses because the blood levels gamma-tocopherol serve as a reflection of metabolic response to oxidative stress and inflammation rather than a reflection of vitamin E intake. We also extended our analyses by assessing the associations of gamma-tocopherol with oxidative balance score (OBS), and several other biomarkers of oxidative stress and inflammation including F2-isoprostanes (FIP), fluorescent oxidation products (FOP), and C-reactive protein (CRP).

Methods:
The research data were obtained from two previous case-control studies of incident, sporadic colorectal adenoma conducted several years apart using similar protocols in two different U.S. states. Gamma-tocopherol levels were categorized into tertile intervals, and OBS was divided into three equal intervals. Gamma-tocopherol and biomarker levels were also dichotomized based on median values among controls. Unconditional logistic regression was used to calculate adjusted odds ratios (ORs) and corresponding 95% Confidence Intervals (95% CIs).

Results:
There were no significant associations between gamma-tocopherol (used as a continuous, dichotomized, or three-level variable) and incident, sporadic colorectal adenoma. In the analysis of the association between OBS and gamma-tocopherol, the OR for the middle vs. lowest OBS interval was 0.57 (95% CI: 0.31- 1.04), and the corresponding OR for the highest vs. lowest interval was 0.17 (95% CI: 0.07-0.41; P trend<0.001). FIP was significantly associated with plasma concentrations of gamma-tocopherol; and adjusted ORs for the medium and high levels of gamma-tocopherol were 1.51 (95% CI: 0.79-2.87) and 3.28 (95% CI: 1.58, 6.80) compared to low gamma-tocopherol concentration. None of the corresponding estimates for FOP and CRP were significantly different from the null value.

Conclusion:
Although gamma-tocopherol was not associated with colorectal adenoma directly, the significant associations between gamma-tocopherol and OBS/FIP indicate that gamma-tocopehrol may be capable of reflecting oxidation levels. A cohort study is needed to further evaluate this issue.

Oxidative Stress, Gamma-tocopherol and Colorectal Adenoma

By

Dina Huang

Bachelor of Science
Huazhong Agricultural University
2013

Thesis Committee Chair: Michael Goodman, MD MPH

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2015

Table of Contents

Oxidative Stress, Gamma-tocopherol and Colorectal Adenoma

Introduction

Oxidative stress is thought to be both an initiator and a promoter of carcinogenesis [1-3]. Despite compelling biological evidence, epidemiologic studies on the associations between determinants of oxidative stress and cancer risk remain inconclusive. An important challenge facing population-based studies of oxidative stress is the relative paucity of biomarkers that can accurately measure oxidation in humans. One biomarker that may serve this purpose is plasma gamma-tocopherol. Gamma-tocopherol is an important isoform of vitamin E that acts as a potent dietary anti-oxidant. More importantly, plasma concentration of gamma-tocopherol increases in response to inflammation and oxidative stress, and thus serves as a marker of these two closely related processes. In this study, we assess the role of gamma-tocopherol, as a measure the oxidative stress burden and its relation to colorectal neoplasia.

Oxidative stress and carcinogenesis

Oxidative stress is defined as an imbalance between pro-oxidant and antioxidant factors in favor of the former resulting in overproduction of reactive oxygen species (ROS)[4]. ROS may stimulate carcinogenesis by inducing mutations, damaging genomic DNA or disrupting cell cycle [4]. Oxidative stress is also closely associated with inflammation.

Inflammatory cells are characterized by excessive production of ROS [5, 6]. Conversely, mediators of inflammation including metabolites of arachidonic acid, cytokines and chemokines are overproduced in the presence of oxidative stress [7].

A substantial body of evidence has supported the role of oxidative stress in initiation, promotion and progression of colon tumors [8]. Prolonged DNA damage initiated by oxidative stress results in arrest or induction of transcription, alteration of signal transduction pathways, replication errors and genomic instability, which are all associated with colon carcinogenesis [4]. In addition, colon cancer is closely associated with inflammation. Previous study has demonstrated that risk of colorectal cancer among patients with inflammatory bowel disease (e.g. ulcerative colitis) is 6 times higher than the corresponding risk in the general population [9]. Excessive production of ROS results in persistent inflammation and the injured cells tend to recruit inflammatory cells and continue to produce ROS [10, 11]. Hence, sustained ROS environment with inflammatory response aggravates prolonged cell injury and lead to colon carcinogenesis.

Markers of oxidative stress and inflammation and their association with colorectal tumors

The three markers previously examined in relation to both colorectal adenoma risk and OBS include F2-isoprostanes (FIP), fluorescent oxidative products (FOP) and C-reactive-protein (CRP).

FIP are considered to be the most established biomarker of oxidative stress [12-14]. However, FIP only measures lipid peroxidation and it does not take into account other aspects of oxidative damage The methodology of measuring FIP is complicated by rather rigorous requirements for proper sample collection and storage to prevent in vitro oxidation. This affects the use of FIP in large epidemiological studies with in-the-field sample collection [15-17].

Unlike FIP, which only measures lipid peroxidation and is relatively unstable, FOP is thought to be a global indicator of oxidative stress that is not affected by *in vitro* oxidation. FOP measures byproducts of oxidation of lipids, proteins and DNA [18]. Historically, FOP has been used to measure oxidation in food, in animals and in vitro studies. More recently, it was proposed as a novel global biomarker of oxidative stress for large epidemiological studies [16]. When interpreting FOP data, however, it is important to keep in mind that some products not related to oxidative stress can also generate fluorescence [17].

As oxidative stress is also closely related to inflammation, studies assessing biomarkers of oxidative stress also tend to include CRP. Serum CRP levels increase in response to inflammatory stimuli and for this reason it is regarded as an established and reliable biomarker of inflammation [19, 20]. Recent studies demonstrate that markers of oxidative stress may also correlate with CRP.

Gamma-tocopherol

Gamma-tocopherol, a major isoform of vitamin E, is a potent dietary antioxidant. Although gamma-tocopherol is the most common form of vitamin E in the American diet, plasma concentrations of gamma-tocopherol are only one tenth of the concentrations of alpha-tocopherol, which is another major isoform of vitamin E [21, 22].

Unlike alpha-tocopherol, which preferentially absorbed by α-tocopherol transfer protein (α-TTP), gamma-tocopherol does not have a specific transfer mechanism [23]. As most of the ingested alpha-tocopherol is found in plasma it serves as an indicator of vitamin E intake. By contrast plasma concentration of gamma-tocopherol is less influenced by dietary intake, but rather serves as a biomarker of endogenous levels of oxidative stress and inflammation.

Both gamma-tocopherol and alpha-tocopherol function as anti-oxidants; however, only gamma-tocopherol and its metabolites possess anti-inflammatory properties [24, 25]. Although gamma- and alpha-tocopherol have similar ROS scavenging capacity [26, 27]; alpha-tocopherol is probably more important because its plasma levels are much higher. On the other hand gamma-tocopherol is more effective in capturing reactive nitrogen radicals [28] and nucleophilic oxidants such as peroxynitrite [29]. In addition to reacting with ROS and nitrogen species, gamma-tocopherol also reduces inflammation and inflammatory damage. It has been reported that gamma-tocopherol and its metabolites inhibit the activity of the cyclooxygenase enzyme, which mediates and further induces inflammation [30].

A number of experimental biology studies argued that that it is the anti-inflammation rather than anti-oxidant properties of gamma-tocopherol and its metabolites that may help reduce colorectal cancer risk [31]. Others have shown that gamma-tocopherol may influence carcinogenesis by inhibiting cell proliferation of colon epitelium [32] and by downgrading cyclin D1 and cyclin E levels [32].

Tocopherols and colon tumors in human population studies

The epidemiological investigations of vitamin E intake showed weak or statistically non-significant associations with colorectal tumors [33-35]. The studies that assessed the association between blood tocopherol levels and colon cancer were also inconsistent with predominantly null results [36-42].

The data on the association between tocopherol levels and adenoma risk are scarce. One study compared plasma alpha- and gamma-tocopherol concentrations among 332 colorectal adenoma patients and 363 sigmoidoscopy-confirmed controls [43]. Higher alpha-tocopherol and lower gamma-tocopherol levels were associated with decreased occurrence of large (at least 1 cm) but not small adenomas; however, the results were no longer statistically significant after adjusting for confounders. A stronger and more consistent trend was observed when exposure of interest was expressed as alpha-tocopherol-to-gamma-tocopherol ratio. These results indicate that combined tocopherol exposure measures may serve as better predictor of decreased cancer risk than high plasma alpha-tocopherol alone.

Oxidative Balance Score

As oxidative stress is thought to be important in the pathogenesis of colorectal tumors, it has been proposed that increased intake of antioxidants (not just tocopherols) may help prevent colorectal adenomas and cancer. However epidemiological studies addressing this hypothesis reported weak and inconsistent associations [33, 34, 44-46]. One possible explanation for these results is the complex and multifactorial mechanisms by which pro- and anti-oxidants affect oxidative stress. Data indicate that a combination of several micronutrients can be more strongly associated with disease risk than any individual agent [47-49]. Previous studies have proposed to address this issue by calculating an oxidative balance score (OBS). OBS is an indicator of oxidative stress-related exposures, which combines various pro- and antioxidant measures into single variable. OBS was found to be associated with colorectal adenoma and with certain markers of oxidative stress [50, 51]; however, data on the associations between OBS and gamma-tocopherol and between gamma-tocopherol and colorectal tumors are lacking.

Methods

Study population

The current analysis used data from two previous case-control studies of incident, sporadic colorectal adenoma. The two studies were conducted by the same principle investigator (Dr. Roberd M. Bostick) in two different US states several years apart.

Both studies were designed to investigate potential biomarkers of risk for incident, sporadic colorectal adenoma. The first study Markers of Adenomatous Polyps I (MAP I) was conducted in Winston-Salem and Charlotte, North Carolina [52, 53]. Participants for the second study Markers of Adenomatous Polyps II (MAP II) were recruited upon referral to Consultants in Gastroenterology, PA, a large private practice in Columbia, South Carolina [54, 55].

To be eligible for the MAP I or MAP II study, participants had to be English speaking, 30-74 years of age, and capable to provide informed consent. Exclusion criteria for both studies were prior history of colorectal adenoma, inflammatory bowel disease, incident colorectal cancer and history of cancers other than non-melanoma skin cancer.

Data collection and laboratory analyses

Data collected for both MAP I and MAP II studies included demographic information, family history, life style and health behaviors, personal body characteristics, and use of medications such as aspirin and other nonsteroidal anti-inflammatory drugs (NSAID).

Dietary information was obtained from the modified 153 item Willett food frequency questionnaire [56, 57].

Blood samples were collected, processed and handled to measure the concentration of pro- or anti-oxidants, FIP, FOP and CRP. The samples were drawn by cold vacutainer tubes and immediately placed on ice. Plasma and serum were separated in a refrigerated centrifuge and placed into O-ring-capped, amber-color cryopreservation vials. MAP I samples were filled with nitrogen in replacement of the air in the vials; MAP II samples were filled with argon. Vials were frozen at -70°C until analysis.

High performance liquid chromatography was used to measure the concentrations of plasma lycopene, alpha-carotene, beta-carotene, lutein, beta-cryptoxanthin, alpha-tocopherol and gamma-tocopherol as described elsewhere [58, 59] Serum ferritin was measured by an antibody-based Roche immunoturbidimetric assay.

Plasma free FIP was measured by gas chromatography-mass spectrometry [60],using deuterium(4)-labeled 8-iso-prostaglandin F_2 alpha as an internal standard. Unlabeled purified FIP was used as a calibration standard.

FOP measurement procedure was modified from the Shimasaki's method [61]. As described elsewhere [62], plasma samples were treated with ethanol-ether and mixed on a vortex mixer. The mixed solution was centrifuged for 10 minutes at 3000 rpm; 1.0mL of supernatant was added to cuvettes for spectrofluorometric readings. The result was expressed in relative fluorescence intensity units per milliliter of plasma at 360/430 nm wavelength [62]. The results of all samples were calculated against 1.0 ppm fluorescent reference standard quinine in 0.1 NH_2SO_4. In approximately one-fifth (22%) of the

samples FOP concentrations were measured by using serum because of the limited amount of plasma samples. In a subset analysis, which used both plasma and serum samples available, the two sets of FOP values were highly correlated ($r=0.9;P<0.001$).

CRP was measured by latex-enhanced immunonephelometry on a Behring nephelometer II (BN-II) analyzer. The interassay coefficient of variation (CV) for CRP was 4%.

Oxidative Balance Score

OBS was calculated by combining the information from a priori selected variables that reflect exposures to pro- and anti-oxidant factors. As described in previous studies [63], levels of micronutrient exposures were categorized as low, medium or high according to study-specific tertile values among controls. The micronutrients measured in plasma concentration included in OBS calculations were lycopene, alpha-carotene, beta-carotene, lutein, beta-crypotexanthin, alpha-tocopherol and serum ferritin. Intakes of polyunsaturated fat and vitamin C were obtained from food frequency questionnaires.

Variables expressing antioxidant exposures were assigned 0, 1, and 2 points, corresponding to the lowest, the middle and the highest tertile, respectively. Conversely, high pro-oxidant exposures were assigned 0 points, medium pro-oxidant exposures received 1 point and low pro-oxidant exposures received 2 points.

For alcohol exposures (measures as number of weekly drinks), heavy drinkers (above median), moderate drinkers (below median) and never drinkers were assigned 0, 1, 2 points respectively. Smoking was categorized as current, former and never and assigned

0, 1, and 2 points respectively. For selenium supplements, and aspirin and NSAID use, no regular use was assigned 0 points, unknown or missing information received 1 point, and regular use was assigned 2 points. The overall OBS score for each participant was calculated by summing the corresponding points.

Statistical analysis

Plasma gamma-tocopherol, the main biomarker of interest in the presents analysis, and OBS (the main exposure variable) were treated as continuous as well as ordinal variables. When treated as an ordinal variable, the OBS distribution was divided into approximately equal intervals, whereas gamma-tocopherol levels were divided into tertiles

We used unconditional logistic regression to assess the associations between the OBS and incident, sporadic adenoma adjusted for age, race, sex, total energy intake, body mass index (BMI), plasma cholesterol, hormone replacement therapy (among women), physical activity, dietary fiber intake, and family history of colorectal cancer in a first degree relative. We then assessed the associations between gamma-tocopherol and the OBS after adjusting for the same potential confounders as in the first model. Next we examined the association between gamma-tocopherol and adenoma controlling for the same covariates. As the candidate of oxidative stress biomarker, gamma-tocopherol was dichotomized based on the study- and sex-specific medians among the controls.

Finally, we assessed the associations between gamma-tocopherol and other biomarkers of oxidative stress (FIP and FOP), controlling for potential confounders. The association between gamma-tocopherol and the marker of inflammation (CRP) was also assessed.

The results of logistic regression analyses were expressed as adjusted odds ratios (ORs) and 95% confidence intervals (CIs). All models were assessed for collinearity and goodness of fit. Statistically significance was defined as a two-sided P value of less than 0.05. SAS version 9.4 (SAS Institute) was used to perform all analyses.

Results

There were 140 cases and 207 controls in the pooled MAP studies that had data on gamma-tocopherol, covariates and all OBS components. Demographic information and some of the characteristics of participants are shown in Table 1. Although cases included higher proportion of males (54% vs. 37% in controls) in terms of other basic demographic factors and health behaviors, cases and controls did not differ significantly in the pooled MAP data. Plasma α-carotene and β-carotene levels were significantly lower in cases, whereas plasma gamma-tocopherol was significantly lower among controls. FIP, FOP and CRP levels were similar in cases and controls.

The OBS ranged from 2 to 24. As shown in table 2, when treating OBS as a continuous variable, the higher score was inversely associated with colorectal adenoma (OR= 0.92, 95% CI: 0.87-0.99). After dividing OBS into equal intervals and using the lowest interval as the reference category, the OR (95% CI) estimates for the middle and the

highest OBS interval were 0.75 (0.42-1.33) and 0.31 (0.14-0.73), respectively (P trend <0.01).

There were no significant associations between gamma-tocopherol (used as a continuous, dichotomized, or three-level variable) and incident, sporadic colorectal adenoma (Table 3). In the analysis assessing the association between OBS and high (above median) gamma-tocopherol level (Table 4), a one-point in the OBS score was associated with a 11% decrease in the likelihood of having high gamma-tocopherol (OR= 0.89, 95% CI: 0.83-0.96). In the analyses that divided OBS into three intervals the OR for the middle vs. lowest OBS interval was 0.57 (95% CI: 0.31- 1.04), and the corresponding OR for the highest vs. lowest interval was 0.17 (95% CI: 0.07-0.41; P trend<0.001).

In the final analysis, we assessed the associations of established markers of oxidative stress and inflammation with gamma-tocopherol. FIP was significantly associated with plasma concentrations of gamma-tocopherol; the adjusted ORs were 2.24 (95% CI: 1.27-3.94, P value: 0.0055). When gamma-tocopherol levels were divided into tertiles, the adjusted ORs for the medium and high levels of gamma-tocopherol were 1.51 (95% CI: 0.79-2.87) and 3.28 (95% CI: 1.58, 6.80) compared to low gamma-tocopherol concentration. None of the corresponding estimates for FOP and CRP were significantly different from the null value.

Discussion

Based on previous studies, we hypothesized that gamma-tocopherol can serve as a biomarker of oxidative stress and may be significantly associated with incident, sporadic colorectal adenoma and with pro- and antioxidant exposures combined into a single score (OBS). The results of our analyses indicated no association between gamma-tocopherol and colorectal adenoma, while OBS was inversely associated with both gamma-tocopherol and adenoma. The oxidative stress biomarker FIP was positively associated with gamma-tocopherol; however, another biomarker FOP and inflammation biomarker CRP were not associated with gamma-tocopherol.

The lack of significant association between gamma-tocopherol and colorectal adenoma is in agreement with other epidemiologic studies that on balance failed to identify a clear link between various tocopherol isomers antioxidants and colorectal neoplasia [42, 45, 64, 65]. A possible explanation is that individual determinants may be involved in complicated biological processes involving complex interactions of multiple pro- / anti-oxidant factors [66]. Because it is difficult to identify the effect of a single determinant, we and others have used OBS as a combined measure of various oxidative stress-related exposures [51, 63, 67, 68].

Although gamma-tocopherol was not associated with colorectal adenoma, the strong and inverse association between gamma-tocopherol and OBS suggested that gamma-tocopherol may indeed serve as a measure of oxidative stress. Two previous studies also

reported that OBS was strongly and inversely associated with FIP, a marker of lipid peroxidation [63, 67].

FIP is considered to be the "gold standard" measure of oxidative stress and FIP was reported to be positively associated with colorectal adenoma[63]. The correlation between gamma-rocopherol and FIP may be explained by high solubility of gamma-tocopherol in blood lipid or by increase in gamma-tocopherol specifically in response to lipid peroxidation[69].

As a biomarker of inflammation, CRP has been linked to oxidative stress [70] and, in at least on previous study, found to be associated with plasma gamma-tocopherol levels [71]. By contrast, our study found little evidence that gamma-tocopherol level was positively associated with serum CRP. The lack of significant association between gamma tocopherol and CRP in our study suggests that these two biomarkers may reflect different aspects of the inflammation-oxidative stress pathway.

A distinguishing feature of this study was the use gamma-tocopherol as a previous unexplored biomarker that may explain the relationship between OBS and colorectal adenoma. Another strength of the study is the use of biomarkers rather than questionnaires to measure OBS components. Food frequency questionnaires may be subject to recall bias while plasma measures serve as better estimates of nutrient intake, absorption and metabolism [72].

Perhaps the most important limitations of the current study are the high proportion of subjects with missing information and the relatively low response rates. In addition the association between OBS and markers of oxidative stress was assessed in a cross-

sectional fashion using the same samples. Prospective follow up studies may be needed to more clearly understand the relationship between oxidative stress-related exposures and the outcomes of interest.

In conclusion, our analysis suggests that blood gamma-tocopherol levels are strongly associated with pro- and anti-oxidant exposures and correlate with blood levels of FIP. However, gamma-tocopherol was not independently associated with colorectal adenoma. As endogenous factors such as antioxidant enzyme activity may greatly affect oxidative balance [4], future studies should take into consideration various oxidative stress-related genetic, epigenetic and phenotypic characteristics that may contribute to the oxidative stress and to the development and progression of colorectal neoplasia.

References

1. Trush, M.A., & Kensler, T. W., *An overview of the relationship between oxidative stress and chemical carcinogenesis.* Free Radical Biology and Medicine, 1991. **10**(3): p. 201-209.
2. Vuillaume, M., *Reduced oxygen species, mutation, induction and cancer initiation.* Mutat Res, 1987. **186**(1): p. 43-72.
3. Wits, G., *Active oxygen species as factors in multistage carcinogenesis.* Experimental Biology and Medicine, 1991. **198**(2): p. 675-682.
4. Klaunig, J.E., and Lisa M. Kamendulis, *The role of oxidative stress in carcinogenesis.* Annu. Rev. Pharmacol. Toxicol, 2004. **44**: p. 239-267.
5. Frenkel, K., *Carcinogen-mediated oxidant formation and oxidative DNA damage.* Pharmacol Ther, 1992. **53**(1): p. 127-66.
6. Shacter, E., et al., *Activated neutrophils induce prolonged DNA damage in neighboring cells.* Carcinogenesis, 1988. **9**(12): p. 2297-304.
7. Hussain, S.P. and C.C. Harris, *Inflammation and cancer: an ancient link with novel potentials.* Int J Cancer, 2007. **121**(11): p. 2373-80.
8. Valko, M., et al., *Free radicals and antioxidants in normal physiological functions and human disease.* Int J Biochem Cell Biol, 2007. **39**(1): p. 44-84.
9. Barrett, C.W., et al., *Tumor suppressor function of the plasma glutathione peroxidase gpx3 in colitis-associated carcinoma.* Cancer Res, 2013. **73**(3): p. 1245-55.
10. Reuter, S., et al., *Oxidative stress, inflammation, and cancer: how are they linked?* Free Radic Biol Med, 2010. **49**(11): p. 1603-16.
11. Gu, Y., et al., *The first characterization of free radicals formed from cellular COX-catalyzed peroxidation.* Free Radic Biol Med, 2013. **57**: p. 49-60.

12. Kadiiska, M.B., et al., *Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning?* Free Radic Biol Med, 2005. **38**(6): p. 698-710.

13. Liu, T., et al., *The isoprostanes: novel prostaglandin-like products of the free radical-catalyzed peroxidation of arachidonic acid.* J Biomed Sci, 1999. **6**(4): p. 226-35.

14. Milne, G.L., E.S. Musiek, and J.D. Morrow, *F2-isoprostanes as markers of oxidative stress in vivo: an overview.* Biomarkers, 2005. **10 Suppl 1**: p. S10-23.

15. Kitano, S., et al., *Improved method of plasma 8-Isoprostane measurement and association analyses with habitual drinking and smoking.* World J Gastroenterol, 2006. **12**(36): p. 5846-52.

16. Wu, T., et al., *Plasma fluorescent oxidation products as potential markers of oxidative stress for epidemiologic studies.* Am J Epidemiol, 2007. **166**(5): p. 552-60.

17. Wu, T., et al., *Plasma fluorescent oxidation products: independent predictors of coronary heart disease in men.* Am J Epidemiol, 2007. **166**(5): p. 544-51.

18. Dillard, C.J. and A.L. Tappel, *Fluorescent damage products of lipid peroxidation.* Methods Enzymol, 1984. **105**: p. 337-41.

19. Ridker, P.M., et al., *Established and emerging plasma biomarkers in the prediction of first atherothrombotic events.* Circulation, 2004. **109**(25 Suppl 1): p. Iv6-19.

20. Ballantyne, C.M. and V. Nambi, *Markers of inflammation and their clinical significance.* Atheroscler Suppl, 2005. **6**(2): p. 21-9.

21. Wolf, G., *How an increased intake of alpha-tocopherol can suppress the bioavailability of gamma-tocopherol.* Nutr Rev, 2006. **64**(6): p. 295-9.

22. Cui, R., Z.Q. Liu, and Q. Xu, *Blood alpha-tocopherol, gamma-tocopherol levels and risk of prostate cancer: a meta-analysis of prospective studies.* PLoS One, 2014. **9**(3): p. e93044.

23. Traber, M.G., *Vitamin E regulatory mechanisms.* Annu Rev Nutr, 2007. **27**: p. 347-62.

24. Cook-Mills, J.M. and C.A. McCary, *Isoforms of vitamin E differentially regulate inflammation.* Endocr Metab Immune Disord Drug Targets, 2010. **10**(4): p. 348-66.

25. Berdnikovs, S., et al., *Isoforms of vitamin E have opposing immunoregulatory functions during inflammation by regulating leukocyte recruitment.* J Immunol, 2009. **182**(7): p. 4395-405.

26. Atkinson, J., R.F. Epand, and R.M. Epand, *Tocopherols and tocotrienols in membranes: a critical review.* Free Radic Biol Med, 2008. **44**(5): p. 739-64.

27. Yoshida, Y., et al., *Chemical reactivities and physical effects in comparison between tocopherols and tocotrienols: physiological significance and prospects as antioxidants.* J Biosci Bioeng, 2007. **104**(6): p. 439-45.

28. Cooney, R.V., et al., *Products of gamma-tocopherol reaction with NO2 and their formation in rat insulinoma (RINm5F) cells.* Free Radic Biol Med, 1995. **19**(3): p. 259-69.

29. Christen, S., et al., *gamma-tocopherol traps mutagenic electrophiles such as NO(X) and complements alpha-tocopherol: physiological implications.* Proc Natl Acad Sci U S A, 1997. **94**(7): p. 3217-22.

30. Hussain, S.P., et al., *Nitric oxide, a mediator of inflammation, suppresses tumorigenesis.* Cancer Res, 2004. **64**(19): p. 6849-53.

31. Jiang, Q., et al., *Gamma-tocopherol attenuates moderate but not severe colitis and suppresses moderate colitis-promoted colon tumorigenesis in mice.* Free Radic Biol Med, 2013. **65**: p. 1069-77.

32. Gysin, R., A. Azzi, and T. Visarius, *Gamma-tocopherol inhibits human cancer cell cycle progression and cell proliferation by down-regulation of cyclins.* Faseb j, 2002. **16**(14): p. 1952-4.

33. Bostick, R.M., et al., *Reduced risk of colon cancer with high intake of vitamin E: the Iowa Women's Health Study.* Cancer Res, 1993. **53**(18): p. 4230-7.

34. Greenberg, E.R., et al., *A clinical trial of antioxidant vitamins to prevent colorectal adenoma. Polyp Prevention Study Group.* N Engl J Med, 1994. **331**(3): p. 141-7.

35. Longnecker, M.P., et al., *Serum alpha-tocopherol concentration in relation to subsequent colorectal cancer: pooled data from five cohorts.* J Natl Cancer Inst, 1992. **84**(6): p. 430-5.

36. Stahelin, H.B., et al., *Cancer, vitamins, and plasma lipids: prospective Basel study.* J Natl Cancer Inst, 1984. **73**(6): p. 1463-8.

37. Nomura, A.M., et al., *Serum vitamin levels and the risk of cancer of specific sites in men of Japanese ancestry in Hawaii.* Cancer Res, 1985. **45**(5): p. 2369-72.

38. Schober, S.E., et al., *Serologic precursors of cancer. I. Prediagnostic serum nutrients and colon cancer risk.* Am J Epidemiol, 1987. **126**(6): p. 1033-41.

39. Wald, N.J., et al., *Serum vitamin E and subsequent risk of cancer.* Br J Cancer, 1987. **56**(1): p. 69-72.

40. Knekt, P., et al., *Serum vitamin E and risk of cancer among Finnish men during a 10-year follow-up.* Am J Epidemiol, 1988. **127**(1): p. 28-41.

41. Comstock, G.W., K.J. Helzlsouer, and T.L. Bush, *Prediagnostic serum levels of carotenoids and vitamin E as related to subsequent cancer in Washington County, Maryland.* Am J Clin Nutr, 1991. **53**(1 Suppl): p. 260s-264s.

42. Kabat, G.C., et al., *Repeated measurements of serum carotenoid, retinol and tocopherol levels in relation to colorectal cancer risk in the Women's Health Initiative.* Eur J Clin Nutr, 2012. **66**(5): p. 549-54.

43. Ingles, S.A., et al., *Plasma tocopherol and prevalence of colorectal adenomas in a multiethnic population.* Cancer Res, 1998. **58**(4): p. 661-6.

44. Murtaugh, M.A., et al., *Antioxidants, carotenoids, and risk of rectal cancer.* Am J Epidemiol, 2004. **159**(1): p. 32-41.

45. Bjelakovic, G., et al., *Antioxidant supplements for preventing gastrointestinal cancers.* Cochrane Database Syst Rev, 2008(3): p. Cd004183.

46. Bjelakovic, G., et al., *Antioxidant supplements for prevention of gastrointestinal cancers: a systematic review and meta-analysis.* Lancet, 2004. **364**(9441): p. 1219-28.

47. Duthie, S.J., et al., *Antioxidant supplementation decreases oxidative DNA damage in human lymphocytes.* Cancer Res, 1996. **56**(6): p. 1291-5.

48. Slattery, M.L., et al., *Eating patterns and risk of colon cancer.* Am J Epidemiol, 1998. **148**(1): p. 4-16.

49. Trichopoulou, A., et al., *Diet and overall survival in elderly people.* Bmj, 1995. **311**(7018): p. 1457-60.

50. Goodman, M., et al., *A summary measure of pro- and anti-oxidant exposures and risk of incident, sporadic, colorectal adenomas.* Cancer Causes Control, 2008. **19**(10): p. 1051-64.

51. Goodman, M., et al., *Hypothesis: oxidative stress score as a combined measure of pro-oxidant and antioxidant exposures.* Ann Epidemiol, 2007. **17**(5): p. 394-9.

52. Boyapati, S.M., et al., *Calcium, vitamin D, and risk for colorectal adenoma: dependency on vitamin D receptor BsmI polymorphism and nonsteroidal anti-inflammatory drug use?* Cancer Epidemiol Biomarkers Prev, 2003. **12**(7): p. 631-7.

53. Gong, Y.L., et al., *Vitamin D receptor gene Tru9I polymorphism and risk for incidental sporadic colorectal adenomas.* World J Gastroenterol, 2005. **11**(31): p. 4794-9.

54. Daniel, C.R., et al., *TGF-alpha expression as a potential biomarker of risk within the normal-appearing colorectal mucosa of patients with and without incident sporadic adenoma.* Cancer Epidemiol Biomarkers Prev, 2009. **18**(1): p. 65-73.

55. Sidelnikov, E., et al., *MutL-homolog 1 expression and risk of incident, sporadic colorectal adenoma: search for prospective biomarkers of risk for colorectal cancer.* Cancer Epidemiol Biomarkers Prev, 2009. **18**(5): p. 1599-609.

56. Willett, W.C., et al., *The use of a self-administered questionnaire to assess diet four years in the past.* Am J Epidemiol, 1988. **127**(1): p. 188-99.

57. Willett, W.C., et al., *Reproducibility and validity of a semiquantitative food frequency questionnaire.* Am J Epidemiol, 1985. **122**(1): p. 51-65.

58. Bieri, J.G. and a.J.C.S.J. Ellen D. Brown, *Determination of individual carotenoids in human plasma by high performance liquid chromatography.* Journal of Liquid Chromatography 1985. **8**(3): p. 473-484.

59. De Leenheer, A.P., et al., *Simultaneous determination of retinol and alpha-tocopherol in human serum by high-performance liquid chromatography.* J Chromatogr, 1979. **162**(3): p. 408-13.

60. Morrow, J.D. and L.J. Roberts, 2nd, *Mass spectrometric quantification of F2-isoprostanes in biological fluids and tissues as measure of oxidant stress.* Methods Enzymol, 1999. **300**: p. 3-12.

61. Shimasaki, H., *Assay of fluorescent lipid peroxidation products.* Methods Enzymol, 1994. **233**: p. 338-46.

62. Wu, T., et al., *Stability of measurements of biomarkers of oxidative stress in blood over 36 hours.* Cancer Epidemiol Biomarkers Prev, 2004. **13**(8): p. 1399-402.

63. Kong, S.Y., et al., *Oxidative balance score, colorectal adenoma, and markers of oxidative stress and inflammation.* Cancer Epidemiol Biomarkers Prev, 2014. **23**(3): p. 545-54.

64. Max, J.B., et al., *IGF-I, IGFBP-3, and IGF-I/IGFBP-3 ratio: no association with incident colorectal cancer in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study.* Cancer Epidemiol Biomarkers Prev, 2008. **17**(7): p. 1832-4.

65. Leenders, M., et al., *Plasma and dietary carotenoids and vitamins A, C and E and risk of colon and rectal cancer in the European Prospective Investigation into Cancer and Nutrition.* Int J Cancer, 2014.

66. Wright, M.E., et al., *Development of a comprehensive dietary antioxidant index and application to lung cancer risk in a cohort of male smokers.* Am J Epidemiol, 2004. **160**(1): p. 68-76.

67. Lakkur, S., et al., *Oxidative balance score and oxidative stress biomarkers in a study of Whites, African Americans, and African immigrants.* Biomarkers, 2014. **19**(6): p. 471-80.

68. Dash, C., et al., *Using pathway-specific comprehensive exposure scores in epidemiology: application to oxidative balance in a pooled case-control study of incident, sporadic colorectal adenomas.* Am J Epidemiol, 2013. **178**(4): p. 610-24.

69. Tanaka, Y., L.A. Wood, and R.V. Cooney, *Enhancement of intracellular gamma-tocopherol levels in cytokine-stimulated C3H 10T1/2 fibroblasts: relation to NO synthesis, isoprostane formation, and tocopherol oxidation.* BMC Chem Biol, 2007. **7**: p. 2.

70. Yasunari, K., et al., *Oxidative stress in leukocytes is a possible link between blood pressure, blood glucose, and C-reacting protein.* Hypertension, 2002. **39**(3): p. 777-80.

71. Cooney, R.V., et al., *Elevated plasma gamma-tocopherol and decreased alpha-tocopherol in men are associated with inflammatory markers and decreased plasma 25-OH vitamin D.* Nutr Cancer, 2008. **60 Suppl 1**: p. 21-9.

72. Potischman, N., *Biologic and methodologic issues for nutritional biomarkers.* J Nutr, 2003. **133 Suppl 3**: p. 875s-880s.

Tables

Table 1. Characteristics of participants in the MAP I and MAP II case-control studies of incident, sporadic colorectal adenoma

Characteristics of study participants	MAP I		MAP II		Pooled analysis	
	Cases(n=106) mean (SD) or %	Controls(n=113) mean (SD) or %	Cases(n=34) mean (SD) or %	Controls(n=94) mean (SD) or %	Cases(n=140) mean (SD) or %	Controls(n=207) mean (SD) or %
Age, y	57.5 (8.9)	56.4 (10.4)	55.3 (7.2)	55.6 (7.8)	57.0 (8.6)	56.0 (9.3)
Male (%)	53.8	31.0[a]	55.9	43.6	54.3	36.7[a]
BMI, kg/m²	27.7 (6.1)	27.1 (5.5)	28.5 (5.2)	28.6 (6.7)	27.9 (5.9)	27.8 (6.1)
Physical activity, MET-hours/week	216.8 (144.6)	195.1 (128.9)	168.0 (117.9)	173.0 (126.1)	204.9 (139.8)	185.1 (127.8)
Family history of colorectal cancer[c] (%)	18.9	34.5[a]	20.6	20.2	19.3	28.0
HRT user (women only; %)	63.0	54.0	78.6	70.6	66.7	60.6
Regularly take an NSAID (%)	21.7	34.5[b]	32.4	34.0	24.3	34.3
Regularly take aspirin (%)	34.9	35.4	47.1	42.6	37.9	38.7
Current smoker (%)	34.0	18.6	23.5	12.8	31.4	15.9
Current drinker (%)	42.5	41.6	76.5	54.3[b]	50.7	47.3
Dietary intakes per day						
Total energy, kcal	2064.9 (861.0)	2177.1 (2456.4)	1837.0 (754.4)	1642.9 (641.8)	2009.6 (839.5)	1934.5 (1880.9)
Total PUFA[d], gm	14.0 (6.4)	14.4 (14.3)	15.6 (8.8)	14.1 (10.3)	14.4 (7.1)	14.3 (12.6)
Dietary fiber, gm	22.9 (9.4)	25.7 (26.2)	16.6 (6.6)	15.4 (6.7)	21.3 (9.2)	21.0 (20.5)
Total[e] vitamin C, mg	281.6 (386.8)	296.5 (341.5)	236.5 (269.5)	294.7 (366.5)	270.7 (361.4)	295.7 (352.2)
Plasma levels						
Plasma lycopene, µg/dL	26.5 (14.2)	25.8 (13.3)	22.1 (11.5)	25.0 (11.3)	25.4 (13.7)	25.4 (12.4)
Plasma α-carotene, µg/dL	2.8 (2.9)	3.6 (4.8)	2.6 (2.6)	3.6 (3.2)	2.8 (2.8)	3.6 (4.1)[b]
Plasma β-carotene, µg/dL	15.4 (22.5)	16.6 (16.1)	12.4 (11.3)	16.5 (12.9)	14.7 (20.4)	16.5 (14.7)
Plasma lutein, µg/dL	16.8 (7.1)	18.2 (10.3)	17.6 (6.1)	15.9 (6.3)	17.0 (6.8)	17.1 (8.8)
Plasma β-cryptoxanthin, µg/dL	6.0 (4.7)	6.8 (5.7)	6.0 (4.0)	8.1 (7.2)	6.0 (4.5)	7.4 (6.4)[b]
Plasma α-tocopherol, mg/dL	1.2 (0.5)	1.1 (0.5)	1.1 (0.3)	1.2 (0.5)	1.1 (0.5)	1.2 (0.5)
Plasma γ-tocopherol, mg/mL	236.7 (111.7)	226.8 (114.7)	209.3 (114.5)	181.9 (92.5)	230.1 (112.6)	206.4 (107.3)[b]
Plasma ferritin, mg/dL	147.3 (135.8)	146.1 (183.1)	141.6 (108.0)	130.1 (126.2)	145.9 (129.2)	138.8 (159.7)
Plasma total cholesterol, mg/dL	203.6 (36.2)	207.5 (40.6)	196.3 (33.1)	200.8 (40.3)	201.8 (35.5)	204.4 (40.5)
Biomarker levels						
FIP, pg/mL	91.8 (38.4)	87.9 (36.1)	74.9 (25.2)	77.6 (28.8)	88.0 (36.5)	83.8 (33.7)
FOP, avg. std. ref. adj.[f]	0.06 (0.14)	0.05 (0.18)	0.03 (0.01)	0.04 (0.01)	0.05 (0.12)	0.05 (0.13)
CRP, µg/mL	5.8 (5.6)	7.5 (23.4)	3.8 (4.9)	4.6 (6.1)	5.3 (5.5)	6.2 (17.8)

a. P<0.01, T test for continuous variables or χ^2 test for categorical variables

b. P<0.05, T test for continuous variables or χ^2 test for categorical variables

c. Family history in a first-degree relative

d. PUFA, abbreviation for polyunsaturated fatty acid

e. Total contains dietary and supplemental intake

f. Average standard reference adjusted

Table2. Association between OBS and incident, sporadic colorectal adenoma

	Cases (n)	Controls (n)	OR (95% CI)[a]	P
OBS without g-t (range,2-24)				
Interval 1(OBS,2-9)	44	43	1.00	<0.01
Interval 2(OBS,10-16)	82	115	0.75 (0.42-1.33)	
Interval 3(OBS,17-24)	14	49	0.31 (0.14-0.73)	
OBS as continuous variable	140	207	0.92 (0.87-0.99)	0.01

a. Adjusted for age, race, sex, BMI, total energy intake, plasma cholesterol, family history in a first-degree relative, hormone replacement therapy(among women), dietary fiber, physical activity, and study (MAPI or MAPII).

Table3. Associations of γ-tocopherol with incident, sporadic colorectal adenoma

Biomarker	Cases (n)	Controls (n)	OR (95% CI)[c]	P
γ-tocopherol (continuous)	140	207	1.00 (1.00-1.01)	0.06
γ-tocopherol (tertile)[a]				
interval 1 (<156 mg/mL)	44	73	1.00	0.15
interval 2 (156-241,mg/mL)	41	74	0.85 (0.47-1.53)	
interval 3 (>241 mg/mL)	55	60	1.60 (0.85-3.01)	
γ-tocopherol (dichotomized)[b]				
low	67	105	1.00	0.54
high	73	102	1.17 (0.71-1.92)	

a. γ-tocopherol intervals are categorized using tertile values.

b. γ-tocopherol was dichotomized according to study- ,sex-specific median among controls.

c. Adjusted for age, race, sex, BMI, total energy intake, plasma cholesterol, family history in a first-degree relative, hormone replacement therapy (among women), dietary fiber, physical activity, and study (MAPI or MAPII).

Table4. Associations between OBS and γ-tocopherol

OBS	γ-tocopherol[a]			
	Low	High	OR (95% CI)[b]	P
interval1 (OBS,2-9)	36	51	1.00	<0.001
interval2 (OBS,10-16)	93	104	0.57 (0.31-1.04)	
interval3 (OBS,17-24)	43	20	0.17 (0.07-0.41)	
continuous	172	175	0.89 (0.83-0.95)	<0.001

a. γ-tocopherol was dichotomized according to study- ,sex-specific median among controls.
b. Adjusted for age, race, sex, BMI, total energy intake, plasma cholesterol, family history in a first-degree relative, hormone replacement therapy (among women), dietary fiber, physical activity, and study (MAP I or MAPII).

Table 5. Association between γ-tocopherol and Biomarkers of Oxidative Stress and Inflammation

Gamma-tocopherol level[c]		Biomarkers[a]		OR (95% CI)[d]	P-value
		High[b]	Low[b]		
		FIP			
tertile	interval 1 (<156 mg/mL)	34	53	1.00	0.0015
	interval 2 (156-241, mg/mL)	52	43	1.51 (0.79-2.87)	
	interval 3 (>241 mg/mL)	73	24	3.28 (1.58-6.80)	
dichotomized	low	59	76	1.00	0.0055
	high	100	44	2.24 (1.27-3.94)	
continuous(log transformed)		159	120	2.58 (1.42-4.69)	0.0019
		FOP			
tertile	interval 1 (<156 mg/mL)	60	52	1.00	0.86
	interval 2 (156-241, mg/mL)	58	48	0.95 (0.53-1.68)	
	interval 3 (>241 mg/mL)	62	44	0.95 (0.51-1.77)	
dichotomized	low	87	73	1.00	0.72
	high	93	71	0.91 (0.56-1.50)	
continuous(log transformed)		180	144	1.01 (0.62-1.67)	0.96
		CRP			
tertile	interval 1 (<156 mg/mL)	49	68	1.00	0.35
	interval 2 (156-241, mg/mL)	67	48	1.64 (0.92-2.92)	
	interval 3 (>241 mg/mL)	71	44	1.31 (0.70-2.45)	
dichotomized	low	81	91	1.00	0.89
	high	106	69	1.04 (0.63-1.70)	
continuous(log transformed)		187	160	1.52 (0.92-2.52)	0.10

a. Biomarkers were dichotomized according to study- ,sex-specific medians among controls.

b. Total numbers of participants differ because of missing biomarker data.

c. Three-intervals γ-tocopherol category is based on tertile values, dichotomized γ-tocopherol category is based on study- ,sex-specific median among controls.

d. Adjusted for age, race, sex, BMI, total energy intake, plasma cholesterol, family history in a first-degree relative, hormone replacement therapy (among women), dietary fiber, physical activity, and study (MAPI or MAPII).