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Elevated plasma γ -tocopherol and decreased α -tocopherol in men are associated with inflammatory markers and decreased plasma 25-OH vitamin D

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Abstract

Chronic inflammation is a risk factor for many diseases of aging. Endogenous oxidants are thought to mediate the effects of inflammation and γ -Tocopherol (γ -Toc) may mitigate damage from nitrogen-based oxidants, however, no physiological requirement for γ -Toc has been established. Regulation of tocopherols and their functional significance are poorly defined, thereby limiting their application in prevention. Using stored plasma samples from 657 male control subjects in a previous study of prostate cancer we have analyzed associations of the tocopherols, inflammation markers, and 25-OH vitamin D. Plasma α -Toc and γ -Toc were inversely correlated, whereas δ -Toc and α -Toc levels were positively correlated, suggesting a unique regulatory mechanism. γ -Toc levels were positively and α -Toc negatively associated with plasma C-reactive protein (CRP) and urinary isoprostane F_{2t} , which are markers of inflammation and oxidation. Ethnic variability in tocopherols was observed, however, this may be explained by differences in plasma 25-OH vitamin D, as γ -Toc levels varied inversely and α -Toc positively with 25-OH vitamin D. In these data all-cause mortality appeared to be positively associated with CRP and inversely with 25-OH vitamin D. We hypothesize that plasma levels of tocopherols may serve as markers of systemic inflammation, complicating epidemiologic assessment of their role in cancer etiology.

BACKGROUND

Vitamin E is recognized as an essential dietary nutrient in humans, whereas its role in the prevention of chronic aging-related diseases is controversial [1]. The most recent report from the Food and Nutrition Board of the Institute of Medicine has defined the recommended dietary intake (RDA) of vitamin E (limited to α -tocopherol only) as the amount of α -tocopherol (α -Toc) necessary (15 mg per day in adults) to maintain plasma α -tocopherol levels of 12 μ M (5.16 μ g/ml) [1]. Using this definition the IOM concluded that “the American public is not vitamin E deficient” as more than 95% of the population meets this criterion. The IOM recommendations primarily address the acute consequences of severe vitamin E deficiency, but do not address the role of vitamin E/tocopherols in the maintenance of optimal health and the prevention of diseases of aging, such as cancer and heart disease, due to lack of irrefutable evidence. Despite considerable evidence from *in vitro* mechanistic, animal, and some epidemiologic and clinical studies suggesting beneficial effects from tocopherols at higher plasma levels and at higher doses than the RDA, definitive human evidence is limited. Indeed the lack of critical fundamental information has limited our ability to determine the function of tocopherols in human health, as exemplified by key research recommendations of the IOM report [1] that describe the need to establish “the determinants of plasma concentrations of α -tocopherol”, and whether “these concentrations (are) regulated?”, as well as finding answers to the question “Does γ -tocopherol have a role in humans?”

γ -Tocopherol (γ -Toc) appears to have important biological functions related to its unique chemical attributes that allow it to preferentially react with nitrogen oxidant species [2,3], and thereby limit cellular damage from the enzymatic generation of NO. NO is a mediator of inflammation [4] and thought to be involved in the etiology of inflammation-associated cancers [5]. Many biological effects of γ -Toc have been observed *in vitro* with potentially important biological and health consequences, including effects on sphingolipid metabolism [6], inflammation [7,8], nuclear peroxisome proliferator-activated receptor [9], neoplastic transformation [10], tumor cell proliferation [11], apoptosis [6,12], natriuresis [13], and immune function [14]. Many important non-antioxidant functions have also been attributed to α -Toc such as growth regulation and regulation of immune function [15]. In addition accumulated evidence points to unique roles for the tocopherols as antioxidants [16] and potentially as signaling agents [17]; however, the relevance of tocopherols to human health beyond minimal levels remains obscure and controversial, as does their relevant mechanism (s) of action.

In particular the role of the tocopherols in chronic inflammation is of great potential significance. γ -Toc and its metabolites, but not α -Toc, possess anti-inflammatory activity [7, 8], and γ -Toc levels are observed to rise in animals and cultured fibroblasts in response to inflammatory signals, whereas α -Toc appears to be a primary reactant against cellular oxidants [18,19]. Chronic inflammation is strongly associated with cancer incidence [5] and may occur as a result of chronic infection such as with hepatitis and liver cancer [20], *heliobacter pylori* and stomach cancer [21], or human papilloma virus and cervical cancer [22]. Chronic systemic inflammation is also associated with fat accumulation and body-mass index [23–24] and may contribute to the long-term health effects associated with obesity. Isoprostanes are considered to be specific measures of inflammation [25] and appear to be formed primarily through the enzymatic generation of NO by the inducible nitric oxide synthase enzyme (iNOS) [26,27], induced by cytokines as part of the inflammatory process. C-reactive protein (CRP) is also considered an indicator of systemic inflammation [28]. While γ -Toc protects cells against the lethal effects of endogenous NO generation [29], it also can enhance cellular NO synthesis [30], thereby enhancing the cellular immune response. As a consequence of these many facets of activity, γ -Toc may have important physiological functions, yet its presence in blood may conceivably also signify bodily dysfunction and disease associated with inflammation.

Vitamin D has also gained considerable attention for its association with decreased mortality [31], as well as decreased cancer incidence [32], and vitamin D deficient individuals may exhibit increased levels of systemic inflammation and decreased immune function [33,34]. Its deficiency may result in chronic infection and inflammation potentially leading to increased risk of cancer and/or death. As a consequence, we hypothesize that vitamin D deficiency may lead to increased levels of inflammation markers and alter concentrations of plasma tocopherols.

Only in the last two decades have epidemiologic studies begun to routinely measure dietary and plasma levels of γ -Toc. Previously α -Toc alone was primarily considered in the design of studies and at most, γ -Toc was only fractionally considered as contributing to total vitamin E status. Recent epidemiologic studies have suggested protective associations for γ -Toc with cancer [35,36] and heart disease [37]; however, studies have not carefully considered interactive effects between the tocopherols, functional mechanisms, or the possibility that optimal levels for the tocopherols may yield a bi-phasic response. The assumption that plasma tocopherol levels reflect dietary intake is valid only in cases of severe deficiency or heavy supplement use [1], hence the physiologic determinants of plasma levels of the tocopherols may be key to understanding their potential mechanisms for either reducing or serving as a marker of chronic disease incidence and death and/or understanding their interactions with other physiologic molecules associated with cancer incidence and mortality.

Because of the unique roles for tocopherols in the inflammatory process and immune defense, we sought to better define their association with other markers of inflammation and risk of death and to test the hypothesis that endogenous tocopherol levels might vary with vitamin D status. Using data collected previously from 657 male control subjects in a study of prostate cancer based on a sample of cases and matched controls in the Multiethnic Cohort (MEC), we have analyzed the associations of γ -Toc with other tocopherols, demographic characteristics, markers of inflammation, and plasma 25-OH vitamin D along with a preliminary assessment of their relation to all-cause mortality. The trends and associations seen in such a large number of individuals can offer insight into the underlying processes that determine micronutrient concentrations and related functions *in vivo* and provide insight into design of future epidemiologic studies to assess their association with cancer and mortality.

MATERIALS AND METHODS

Study samples

The Multiethnic Cohort (MEC) is a prospective study that was designed to provide data on diet and other lifestyle exposures related to cancer risk [38]. Funding for the data collection, maintenance and follow-up activities of the MEC is provided through an R37 grant (CA 54281, LN Kolonel, PI). Under two separate grants (P01 CA 33619, LN Kolonel, PI and R01 CA 63464, B.E. Henderson, PI), funding was provided to create a biorepository of blood and urine specimens for the MEC. With the success of both endeavors, a combined prospective biorepository was created, consisting of > 67,000 participants with 40 cc of blood preserved in multiple 0.5 cc aliquots of serum, plasma, red blood cells, and buffy coat stored in liquid nitrogen on each subject.

For this study, samples in the biorepository from 657 men serving as controls for the cases in a study of prostate cancer (L.N. Kolonel NIH Grant 5R37 CA054281) were used. Control subjects were alive with no history of prostate cancer at the age of the case's diagnosis. In addition, controls were matched to cases on area (Hawaii, Los Angeles), ethnicity, age at phlebotomy (within ± 1 year), date of specimen collection (± 1 month), time of day that blood was drawn (± 2 hours), and hours fasting (± 2 hours). Forty-three deaths occurred among these controls in the interval between blood collection and December 31 2005. Deaths were identified from death files from HI and CA and from the National Death Index.

Analytes measured as part of the prostate study included: α -, δ -, and $\beta + \gamma$ -tocopherols (referred to subsequently as γ -Toc, since β -tocopherol represents a very small fraction of the total and co-elutes with γ -Toc), CRP, and a lipid panel. Other data available from the MEC included detailed information [39] on diet, body weight and height, demographic factors (including ethnicity), lifestyle practices (including smoking and physical activity), history of medical conditions, use of medications (including aspirin), use of dietary supplements (including multivitamins), and a family history of common cancers.

Analytical Methods—Analysis of lipid-phase micronutrients from plasma or serum was carried out by HPLC with photo diode array detection [40]. As in past studies, this assay was regularly validated during the analyses for this study for tocopherols through inclusion of external standards in each batch of analysis and by participation in quality assurance programs organized by the US National Institute of Standards and Technology (NIST, Gaithersburg, MD) [41].

In brief, all procedures prior to storage of extracted analytes in amber vials were carried out under subdued light to avoid degradation of analytes. All solvents and serum/plasma samples were kept on ice during work-up. An aliquot of 0.30 mL of plasma was mixed with 0.30 mL ethanol containing butylated hydroxytoluene as antioxidant and 3 internal standards (tocol,

retinyl laurate, and n-butyl- β -apo-8'-carotenoate) followed by partitioning into 0.8 mL hexane [42]. The hexane layer was evaporated in amber vials at room temperature under a stream of nitrogen. The dry extracts were dissolved in 0.15 mL mobile phase of the HPLC system for carotenoids. Inter-assay variabilities of these analytes are between 3 and 9%. HPLC analysis of carotenoids and α -, β -, γ -, and δ -tocopherols was performed on a RP-HPLC system consisting of a Spherex ODS analytical and guard column (150 mm $\times$ 3.2 mm i.d., 3 μ m and 4 mm $\times$ 3 mm i.d., 10 μ m respectively; Phenomenex, Torrance, CA) and a mobile phase consisting of MeOH: CHCl₃: MeCN 665:218:117 (v/v/v) containing 2 mL/L Bis-trispropane (0.5 M; pH 6.8 with HCl) and 0.025% BHT(250 mg/L), which is kept at a flow rate of 0.3 mL/min. PDA detection was performed between 220 – 600 nm with quantitation of tocopherols at 295 nm [42]. Tocol, added to all extracts, was used as an internal standard to adjust tocopherol levels. Injection volumes of 20 μ L from the re-dissolved (0.15 mL) extract was used for HPLC analysis. Plasma C-reactive protein (CRP) was measured according to the manufacturer's directions on a Roche Cobas Mira Plus CC benchtop analyzer (Roche Diagnostics, Ltd., Rotkreutz, Switzerland).

Urinary 15-isoprostane F_{2t} Analysis: 15-isoprostane F_{2t} was measured utilizing a competitive enzyme-linked immunosorbent assay (ELISA) kit from Oxford Biomedical Research, Inc., Oxford, MI (Catalogue # EA85). Urine samples were thawed and mixed with 4 μ L glucuronidase (250,000 units/mL), Oxford Biomedical Research, Inc, Oxford, MI (Catalogue # GL85) and incubated for two hours @ 37 °C and then centrifuged for 2 minutes at 2,000 rpm in a microfuge. Standards and samples (100 μ L) are added in duplicate to 96 well plates, followed by addition of 100 μ L of diluted F_{2t} HRP conjugate and incubated for two hours at room temperature. After washing to remove any unbound substances 200 μ L of substrate solution is added to each well and color allowed to develop proportionate to the amount of isoprostane present. The color development is stopped with the addition of 50 μ L 3N H₂SO₄, and the microplate is then read at 450 nm and also at 590 nm as a background control. Plots of log concentration vs absorbance for standards are prepared and concentrations of unknown samples extrapolated from the standard curve using a four parameter fit and adjusted for any dilution of urine and reported as pg/mL. After measurement of urinary creatinine, isoprostane is then calculated and reported as ng/mg creatinine.

Plasma 25-OH Vitamin D Analysis: *Plasma 25-hydroxyvitamin D (as the sum of 25-hydroxyvitamin D₂ + 25-hydroxyvitamin D₃)* was measured according to the manufacturer's directions utilizing an immunoassay kit purchased from Immunodiagnostic Systems, Ltd., Fountain Hills, AZ (enzymatic kit AA-35F1).

Data Analysis: Mean levels of biomarkers were compared by analysis of variance, after log transformation. The association between biomarkers was examined in a number of ways. Spearman's rho was used as a measure of correlation. Linear regression models of the log-transformed biomarkers on tocopherol levels were performed where the tocopherol levels were categorized into sextiles. The cutpoints were based on the distribution of controls. Geometric mean values and 95% confidence intervals were for correlated each sextile. Additionally, the log-transformed biomarkers were regressed on a trend variable for tocopherol, assigned the median of each sextile. The p-value was used as a measure of dose-response.

Relative risks (RR) of death (all causes) were computed by Cox regression, with age as the time metric. Observation began at blood and urine collection and the earliest of the death date or December 31, 2005. The independent variables were represented as dummy variables representing quintiles and as trend variables. Hazard ratios and 95% confidence intervals were computed as measures of association.

RESULTS

Plasma samples from the 657 control subjects were measured for the analytes listed in Table 1 (Mean age at time of blood draw = 68.8 years; range = 49–84 years). Urinary 15-isoprostane F_{2t} levels were also determined in 416 subjects for which overnight or first morning urines were available as described in Methods. Mean plasma levels of the tocopherols were 16.43 $\mu\text{g/mL}$ (87.6%), 1.98 $\mu\text{g/mL}$ (10.6%), and 0.35 $\mu\text{g/mL}$ (1.88%) for α -, β +, γ -, and δ -tocopherols, respectively, with a mean α/γ -Toc ratio of 15.5 (median = 8.12). Ethnic differences in mean biomarker levels were observed with Caucasians and Japanese exhibiting higher α -Toc and lower γ -Toc levels relative to African Americans and Latinos in this population (Table 2).

Stratification of subjects by sextile of plasma α -Toc level showed a strong inverse association between α -Toc and γ -Toc (Figure 1), whereas, plasma δ -Toc showed a strong positive association with increasing plasma α -Toc, clearly distinguishing δ -Toc from any regulatory mechanism responsible for the decrease in plasma γ -Toc associated with increased α -Toc in this population. The trends were highly significant with p 's < 0.0001. Spearman's rho for gamma tocopherol and alpha tocopherol was -0.40 and for gamma tocopherol and delta tocopherol was 0.19 . Rho between for alpha tocopherol and alpha tocopherol was 0.35 . The relationship between α -Toc and γ -Toc was similar across ethnic groups (data not shown).

Plasma γ -Toc levels were positively associated with urinary 15-isoprostane F_{2t} levels, measured by ELISA and adjusted to urinary creatinine, where p for trend is 0.038 and Rho = 0.13, as well as with C-reactive protein (CRP), where p for trend is 0.001 and Rho = 0.14 (Figure 2). In contrast, α -Toc was inversely associated with each of these markers of inflammation (Figure 3), with Rho = -0.20 for isoprostane F_{2t} (p for trend < 0.0001) and -0.15 for CRP (p for trend = 0.0007).

Smoking is associated with increased inflammation and in the current study current smokers at baseline were observed to have significantly elevated plasma γ -Toc (mean of 2.3 $\mu\text{g/mL}$ for smokers and 1.9 $\mu\text{g/mL}$ for non-smokers, p for t-test = 0.01) and decreased α -Toc (mean of 14.1 $\mu\text{g/mL}$ for smokers and 16.8 $\mu\text{g/mL}$ for non-smokers, p for t-test = 0.01) as shown in Table 3.

Plasma 25-OH vitamin D levels were measured for each of the subjects and showed an inverse relation (p for trend < 0.0001, Rho = -0.24) with plasma γ -Toc level (Figure 4A) and a positive association (p for trend = < 0.0001, Rho = 0.23) with plasma α -Toc (Figure 4B). Separation of the populations by area of collection (Hawaii vs California) indicated that there were significant differences in the vitamin D levels of the two populations, however the relation between γ -Toc and 25-OH vitamin D remained consistent, with all ethnic groups showing a correlation between hyper γ -tocopherolemia (defined as plasma γ -Toc > 2.5 $\mu\text{g/mL}$) with plasma 25-OH vitamin D (Figure 5). Plasma γ -Toc levels in African Americans were inversely related to plasma 25-OH vitamin D (Rho = -0.25) as were those for Latinos (Rho = -0.30), whereas there was a much weaker relationship observed for Caucasians (Rho = 0.06) and Japanese (Rho = 0.006), who had generally higher plasma 25-OH vitamin D levels, suggesting that the association of γ -Toc with 25-OH vitamin D may be manifested principally under conditions of vitamin D deficiency.

The inflammatory marker CRP was significantly positively related to mortality after blood collection in this sample (p for trend = 0.003), with an elevated risk for the fifth quintile (RR = 1.93, 95% CI = 0.84–4.39). Plasma 25-OH vitamin D level was inversely associated with mortality (p for trend = 0.004) with a significantly lower risk for those in the highest quintile for vitamin D (RR = 0.17, 95% CI = 0.04–0.76). There was a suggestion of a weak positive

relationship between risk of death and gamma-tocopherol ($p=0.09$) with a RR of 2.20 (95% CI 0.89–5.42) in the highest quintile of γ -Toc. Isoprostane F_{2t} was not related to risk of death.

DISCUSSION

It is commonly assumed that plasma levels of the tocopherols are determined by their relative binding affinity for the liver tocopherol binding protein [43]. While this may be true for the gross average partitioning of tocopherols into the bloodstream and is consistent with the average levels of tocopherols found in blood for the current study (88% α -Toc, 11% γ -Toc, and 2% δ -Toc), it does not account for the individual variability of plasma levels, whereby we observe a range in the ratio of α/γ -tocopherols between 1 and 252. Although it is known that α -Toc and γ -Toc are inversely related [44] and that supplementation of α -Toc at high levels significantly reduces plasma γ -Toc and δ -Toc concentrations [45], it is not clear that the liver binding protein is the sole means by which plasma concentrations are regulated. In particular the inverse plasma association between α -Toc and γ -Toc among individuals in the current study would appear to be regulated independently of any mass action effect mediated through the liver binding protein. If the inverse relationship were determined solely by increased competition for binding by α -Toc, then one would predict that δ -Toc would be similarly repressed in response to higher α -Toc levels, yet the opposite is observed (Figure 1). In tissue culture we observe a the ratio of γ -Toc to α -Toc is increased in fibroblasts in response to inflammatory cytokines [27], while similarly in rats, increased γ -Toc levels are observed in response to an inflammatory stimulus [18], also suggesting a regulatory mechanism distinct from that of the liver binding protein. In this regard, the study by Gao, et al. [46] is intriguing in that they demonstrated γ -Toc enhances cellular uptake of α -Toc by macrophages, suggesting that higher plasma levels of γ -Toc might decrease plasma α -Toc through increased cellular sequestration. The absence of a strong relationship between tocopherol consumption from food and plasma tocopherol concentration, as summarized previously [3], further supports the hypothesis that tocopherol levels may be physiologically regulated, although clearly supplementation with large doses of α -Toc will significantly depress γ -Toc and raise γ -Toc levels.

Conversion of γ -Toc to various metabolites [47] by cytochrome p450 3A [48] or other enzymes may be an important mechanism controlling γ -Toc levels *in vivo*, as inhibition of the p450 enzyme by sesame lignans can increase plasma γ -Toc concentrations and paradoxically increase α -Toc as well [49]. Recently it was reported that metabolites of γ -Toc were significantly reduced in colon cancer patients compared to controls [47].

Inflammation may be a major determinant in the relative proportion of the tocopherols found in plasma and cells. The positive association observed between plasma γ -Toc and markers of inflammation along with the strong inverse associations observed between plasma α -Toc and inflammatory markers are consistent with previous *in vitro* studies [18,27], as well as evidence that γ -Toc functions as an anti-inflammatory agent [7,8] and that α -Toc is principally consumed by endogenous oxidant generation [27]. Recent studies using mixed tocopherols suggest that a combination of α -Toc and γ -Toc may be optimal in reducing inflammation [50,51]. Smoking also is associated with increased plasma γ -Toc levels in humans [52], a result also seen in the current study. *In vitro* studies are consistent with a model in which γ -Toc possesses unique beneficial chemical and biological properties, balanced by potential negative properties at significantly higher levels. α -Toc also may have important functions distinct from the other tocopherols and is maintained at higher concentrations without apparent negative effects on cells [30]. It may only be under conditions of extreme duress that significantly higher levels of γ -Toc are permitted and potentially required. The inverse relationship between α -Toc and γ -Toc complicates any interpretation of their respective function(s) *in vivo* and whereas α -Toc has been much studied, the role of γ -Toc may have been underestimated in the past. Hyper γ -

tocopherolemia may be an appropriate response to stress and its presence may therefore be an indicator of underlying pathology. Understanding the mechanism by which such a process occurs will be essential to elucidating the tocopherols' physiologic role in maintaining optimal health.

The associations observed with plasma 25-OH vitamin D level and tocopherols are intriguing in light of many studies linking vitamin D to reduced mortality [31], as well as published reports from a large cohort study [53] that showed a significant protective association for α -Toc with subsequent mortality. In that study a U-shaped relationship between baseline plasma α -Toc, adjusted for cholesterol, and all-cause mortality was observed with the lowest mortality rate observed for men with plasma α -Toc levels of approximately 13 – 14 $\mu\text{g/ml}$. Interestingly it is approximately at this plasma level of tocopherol that we begin to see significant depression of γ -Toc levels with increasing plasma α -Toc (Figure 1). The previous study concluded that “higher circulating concentrations of α -Toc within the normal range were associated with significantly lower total and cause-specific mortality” [53]. Although no measurements of γ -Toc were reported for that study, the reported α -Toc levels associated with minimal mortality would be consistent with γ -Toc levels in the moderate range in the present study. Although the number of deaths in the current study is limited, the data indicating a significant positive association between plasma CRP and subsequent mortality support a role for inflammation and further suggest that 25-OH vitamin D levels may be an important marker, if not modifier, of risk and plasma tocopherol levels. Future studies looking at larger numbers of individuals should investigate the potential association of 25-OH vitamin D with tocopherols and their ratios as well as their associations with clinical outcome and/or mortality.

A strength of this study is the inclusion of a number of biomarkers of inflammation and micronutrient levels measured in a relatively large number of men. A limitation, however, is that the sample was not representative of the broader population, but rather was based on controls matched to prostate cancer cases. However, when we compared the associations among ethnic and age groups, most were consistent, implying that the findings may be robust.

The initiation of large clinical studies of high dose α -Toc supplementation were based in part upon an underlying assumption that α -Toc is protective and that raising plasma levels artificially with doses normally not achievable through the diet would be beneficial. Results thus far suggest that supplementation may increase cardiovascular mortality [54–56], although low dose supplementation of α -Toc is associated with decreased mortality [57]. Given the suppressing effect of large supplemental doses on γ -Toc and δ -Toc [45], many scientists have argued against the wisdom of such trials. The results presented in the current study suggest that the tocopherols may be intimately involved in various physiologic and pathologic processes and that interactions with other micronutrients may be important in determining physiologic levels. Indeed with our knowledge of the mechanism of action for γ -Toc as an anti-inflammatory and the tocopherols as specific and unique antioxidants, it would seem more likely that plasma tocopherol levels are generally responsive to underlying conditions rather than causative of those conditions. While clinical data demonstrating decreased prostate cancer incidence and mortality in Finnish male smokers for low dose (50 mg/day) α -Toc [57] support the role of α -Toc in the prevention of cancer, the use of plasma levels as a marker of intake and/or causative risk may require more sophisticated analysis, however. It is likely that tocopherol levels in plasma change in response to pathologic conditions and therefore they may be excellent indicators of potential physiologic abnormalities and risk of death at the population level. However, we need to understand their regulation better before we can determine the feasibility of beneficial interventions. Previously, Ingles et al. reported a significant association between the α/γ -Toc ratio and colorectal adenoma incidence [58]. Future studies of the epidemiology of the tocopherols, as well as clinical intervention trials should assess this novel marker to determine its utility as a marker of risk, as the ratio may be a more

sensitive marker of inflammatory state. Additionally, studies in the basic science of tocopherol regulation are needed prior to designing meaningful intervention trials to optimize human health and longevity.

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Figure 1.

Variation of plasma γ -Toc and δ -Toc levels with plasma α -T levels. Subjects ($n = 657$) were stratified by sextile of plasma α -Toc and the geometric mean for γ -Toc and δ -Toc plotted as a function of median plasma α -Toc level $\pm$ 95% CI.

*Hexiles for α -Toc ($\mu\text{g/ml}$) were ≤ 9.54 ; 9.55–11.77; 11.78–14.38; 14.39–17.43; 17.44–23.26; >23.26

** Based on a linear regression of the variable of interest on a trend variable for α -Toc assigned the median of the sextiles. p value for trend of γ -Toc as a function of α -Toc < 0.0001 ($r^2 = 0.14$); p value for trend of δ -Toc as a function of α -Toc < 0.0001 ($r^2 = 0.12$)

Figure 2.

Variation in markers of inflammation with plasma γ -Toc. Subjects ($n = 657$) were stratified by sextile* of γ -Toc and the median plasma level of CRP and urinary 15-Isoprostane F_{2t} plotted as a function of the geometric mean plasma tocopherol level. Plasma CRP and 15-Isoprostane F_{2t} were determined as described in the methods section.

* Sextiles for γ -Toc ($\mu\text{g/mL}$) were ≤ 0.7421 ; $0.7422-11.1787$; $1.1788-1.6648$; $1.6649-2.2464$; $2.2465-3.1737$; >3.1737 .

** Based on a linear regression of the variable of interest on a trend variable for γ -Toc assigned the median of the sextiles. p value for trend of CRP as a function of γ -Toc < 0.001 ($r^2 = 0.02$); p value for trend of 15-Isoprostane F_{2t} as a function of γ -Toc < 0.038 ($r^2 = 0.01$)

Figure 3.

Variation in markers of inflammation with plasma α -Toc. Subjects ($n = 657$) were stratified by sextile* of α -Toc and the median plasma level of CRP and urinary 15-Isoprostane F_{2t} plotted as a function of the geometric mean plasma tocopherol level. Plasma CRP and 15-Isoprostane F_{2t} were determined as described in the methods section.

* Sextiles for α -Toc ($\mu\text{g/ml}$) were ≤ 9.54 ; 9.55–11.77; 11.78–14.38; 14.39–17.43; 17.44–23.26; >23.26 .

** Based on a linear regression of the variable of interest on a trend variable for α -Toc assigned the median of the sextiles. p value for trend of CRP as a function of α -Toc < 0.0007 ($r^2 = 0.02$); p value for trend of 15-Isoprostane F_{2t} as a function of α -Toc < 0.0001 ($r^2 = 0.04$).

Figure 4.

Association of plasma tocopherols with 25-OH vitamin D. Subjects (n = 657) were stratified by sextile of plasma 25-OH vitamin D and the geometric mean for γ -Toc and α -Toc $\pm$ 95% CI plotted as a function of median plasma 25-OH vitamin D.

*Sextiles for plasma 25-OH vitamin D (nM) were ≤ 50.5 ; 50.6–65.0; 65.1–77.5; 77.6–90.8; 90.9–110.9; >110.9

** Based on a linear regression of the variable of interest on a trend variable for 25-OH vitamin D assigned the median of the sextiles. p value for trend of γ -Toc as a function of 25-OH vitamin D < 0.0001 ($r^2 = 0.05$); p value for trend of α -Toc as a function of 25-OH vitamin D < 0.0001 ($r^2 = 0.05$)

Figure 5.

Association of plasma 25-OH vitamin D with elevated γ -tocopherol by ethnicity. Subjects were stratified first by ethnicity - African Americans (272) and Latinos (104), Japanese/Hawaiians (171) and Caucasians (110) and secondarily by quartile (African Americans) or tertile (Whites, Latinos, and Japanese/Hawaiians) of plasma 25-OH vitamin D for each ethnic group. Subjects were assessed for hyper γ -tocopherolemia (defined as plasma levels of γ -Toc > 2.5 μ g/mL) and the odds ratio determined relative to African Americans in the lowest quartile of plasma 25-OH vitamin D (37 out of 68 had γ -Toc levels > 2.5 μ g/mL) and plotted as a function of median 25 OH-vitamin D for each tertile/quartile within each ethnic group.

Table 1

Characteristics of controls in a study of prostate cancer in the MEC.

	Control Subjects (n = 657)
Mean Years of follow-up	5.1 ± 2.4
Mean Age at Draw (Years)	68.7 ± 7.2
BMI (kg/m ²)	26.3 ± 4.0
Plasma α -Tocopherol (μ g/mL)	16.43 ± 8.46
Plasma γ -Tocopherol (μ g/mL)	1.98 ± 1.36
Plasma δ -Tocopherol (μ g/mL)	0.35 ± 0.16
Plasma α/γ -Tocopherol Ratio	15.5 ± 17.7
Plasma 25-OH Vitamin D (nM)	82.74 ± 37.77
C-Reactive Protein (mg/L)	1.75 ± 2.78
15-Isoprostane F _{2t} (ng/mg Creatinine)	3.79 ± 1.71

Statistics are Mean ± SD unless otherwise stated.

Table 2

Biomarker mean levels by ethnicity of controls in a study of prostate cancer in the MEC.

Analytes	African Americans (n = 272)	Hawaiians (n = 23)	Japanese (n = 148)	Latinos (n = 104)	Caucasian (n = 110)	P for homogeneity [*]
Plasma α -Tocopherol ($\mu\text{g/mL}$)	13.54 $\pm$ 8.47	15.66 $\pm$ 5.52	20.57 $\pm$ 10.36	15.42 $\pm$ 7.97	19.18 [*] $\pm$ 8.85	<0.0001
Plasma γ -Tocopherol ($\mu\text{g/mL}$)	2.22 $\pm$ 1.37	2.49 $\pm$ 1.40	1.54 $\pm$ 1.15	2.16 $\pm$ 1.28	1.67 $\pm$ 1.48	<0.0001
Plasma δ -Tocopherol ($\mu\text{g/mL}$)	0.31 $\pm$ 0.14	0.40 $\pm$ 0.15	0.43 $\pm$ 0.20	0.32 $\pm$ 0.14	0.38 $\pm$ 0.14	<0.0001
α/γ -Tocopherol Ratio	11.44 $\pm$ 14.35	9.44 $\pm$ 8.73	21.79 $\pm$ 21.49	12.54 $\pm$ 15.09	21.14 $\pm$ 19.62	<0.0001
Plasma 25-OH Vitamin D (nM)	65.11 $\pm$ 25.93	93.51 $\pm$ 38.61	93.87 $\pm$ 34.12	77.67 $\pm$ 23.25	114.21 $\pm$ 50.6	<0.0001
Plasma CRP (mg/L)	2.27 $\pm$ 3.12	1.85 $\pm$ 4.30	0.90 $\pm$ 1.70	1.95 $\pm$ 2.86	1.41 $\pm$ 2.26	<0.0001
Urinary 15-Isoprostane $\text{F}_{2\text{a}}$ (ng/mg Creatinine)	3.71 $\pm$ 1.45	3.14 $\pm$ 0.73	3.63 $\pm$ 1.46	3.98 $\pm$ 2.07	4.10 $\pm$ 2.15	0.2334

Statistics are Mean $\pm$ SD unless otherwise stated.

^{*} From analysis of variance of log biomarkers

Table 3

Mean biomarker levels by baseline smoking status.

Analyte	Never-Smoker (n = 200)	Former Smoker (n = 372)	Current Smoker (n = 82)	p for homogeneity
Plasma α -Tocopherol ($\mu\text{g/mL}$)	16.32 $\pm$ 7.61	16.99 $\pm$ 8.76	14.10 $\pm$ 8.88	0.0009
Plasma γ -Tocopherol ($\mu\text{g/mL}$)	1.93 $\pm$ 1.27	1.92 $\pm$ 1.41	2.33 $\pm$ 1.33	0.0135
Plasma δ -Tocopherol ($\mu\text{g/mL}$)	0.36 $\pm$ 0.16	0.36 $\pm$ 0.16	0.33 $\pm$ 0.16	0.0004
Plasma α/γ -Tocopherol Ratio	14.81 $\pm$ 15.09	16.73 $\pm$ 19.03	11.59 $\pm$ 17.29	0.1903
Plasma CRP (mg/L)	1.27 $\pm$ 2.24	1.94 $\pm$ 3.07	2.15 $\pm$ 2.46	0.0001
Urinary 15-Isoprostane F_{2t} (ng/mg Creatinine)	3.78 $\pm$ 1.72	3.73 $\pm$ 1.63	4.20 $\pm$ 2.18	0.3451
Plasma 25-OH vitamin D (nM)	84.88 $\pm$ 37.76	84.34 $\pm$ 38.73	70.88 $\pm$ 31.37	0.0011

Statistics are Mean $\pm$ SD unless otherwise stated.

* From analysis of variance of log biomarkers