Antioxidants and coronary artery disease among individuals with type 1 diabetes: Findings from the Pittsburgh Epidemiology of Diabetes Complications Study


Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15213, USA
Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA 15213, USA

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Abstract

Objective: Oxidative stress has been implicated in the development of diabetes and cardiovascular disease. We evaluated the effect of serum antioxidants and total antioxidant reserve (TAR) on coronary artery disease (CAD) incidence in type 1 diabetes. Methods: Subjects were identified from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC) cohort, a 10-year prospective study of childhood-onset type 1 diabetes. Mean age at baseline was 28 and diabetes duration 19 years. Coronary artery disease was defined as physician-diagnosed angina, confirmed MI, stenosis ≥50%, ischemic electrocardiogram (ECG), or revascularization. Controls were gender, age, and diabetes duration (±3 years) matched with cases. Samples and risk factors used in analyses were identified from the earliest exam prior to incidence in cases (54 cases, 67 controls). Results: None of the antioxidant measures (β-tocopherol, γ-tocopherol, retinol, TAR) showed protection against incident CAD overall. However, a protective effect of α-tocopherol against CAD was observed among antioxidant supplement users (HR=0.22, 95% CI=0.10–0.49) and in renal disease (HR=0.46, 95% CI=0.23–0.91). Despite similar α-tocopherol concentration, there was no protective effect among nonusers of antioxidant supplements. Conclusions: High α-tocopherol levels among patients with renal disease and in those using vitamin supplements were associated with lower CAD risk in type 1 diabetes. The specificity of these effects merits further investigation.

Keywords: Antioxidants; Coronary artery disease; Type 1 diabetes; α-Tocopherol

1. Introduction

Individuals with diabetes mellitus are at increased risk for cardiovascular morbidity and mortality compared to the general population (Haffner et al., 1998). Patients with type 1 diabetes exhibit a risk of cardiovascular mortality up to 10 times higher than nondiabetic subjects (Portuese & Orchard, 1995). Concentrations of atherogenic lipids do not appear to be entirely responsible for this increased risk, since type 1 diabetes patients under adequate glycemic control generally present a relatively normal lipoprotein profile (The Diabetes Control and Complication Research Group, 1992). Oxidative stress, the imbalance between oxidant and antioxidant species, has been implicated in the development of numerous chronic diseases, including diabetes and cardiovascular disease (Baynes & Thorpe, 1999; Matteucci & Giampietro, 2000; West, 2000). Furthermore, it has been suggested that excess production of free radicals and/or potentially decreased antioxidant defenses may contribute to the higher incidence of vascular disease observed in diabetes (Kopprasch et al., 2002; Portuese & Orchard, 1995; Marra et al., 2002). If this hypothesis is true, then increased intake for antioxidants may be beneficial for patients with type 1 diabetes. However, the effectiveness of antioxidants in reducing the risk of CAD in type 1 diabetes has not been well studied. In this study, we evaluated the effect of serum antioxidants and total antioxidant reserve (TAR) on coronary artery disease (CAD) incidence in type 1 diabetes.
of, or treatment with, antioxidant vitamins could prove beneficial in reducing susceptibility to vascular disease in these patients.

However, study findings do not agree on the role of dietary intake or levels of antioxidant vitamin biomarkers in the development or progression of atherosclerosis. Most observational studies suggest a potential protective effect of antioxidant supplementation, although there is confusion as to whether dietary intake from food, pharmacologic supplementation, or both, are of importance. Clinical trials, on the other hand, have generally not supported a protective effect of antioxidant nutrients on cardiovascular outcomes. The few studies conducted among persons with diabetes have also provided conflicting results.

Some of the disagreement among observational studies may be due to differences in the populations studied and the definitions of the outcomes. The discrepancies among clinical trial findings may further relate to the variety of single plasma antioxidants or combinations studied, the supplementation doses, the frequency of use and duration of the treatment regimen, as well as whether the supplements were taken with a meal to optimize absorption. Numerous reports, written to provide a rationale for the contradictory findings among randomized trials (Diaz, Frei, Vita, & Keaney, 1997; Gaut & Heinecke, 2001; Heinecke, 2001; Kaul, Devaraj, & Jialal, 2001; Neuzil, Weber, & Kontush, 2001; Parthasarathy, Khan-Merchant, Penumetcha, Khan, & Santam, 2001; Ricciarelli, Zing, & Azzi, 2001; Tribble, 1999; Witztum & Steinberg, 2001), also give some insight as to why there are discrepancies between observational studies and clinical trials.

The aim of the present examination was to evaluate the effect of serum antioxidants and total antioxidant reserve (TAR) on the incidence of coronary artery disease (CAD) in type 1 diabetes.

2. Methods

Participants were identified from the Pittsburgh Epidemiology of Diabetes Complications Study (EDC) cohort, a 10-year prospective follow-up study of childhood-onset (<17 years of age) type 1 diabetes mellitus. Subjects were first seen in 1986–1988 and were reexamined biennially for 10 years. The EDC study has been previously described (Orchard, Dorman, Maser, Becker, Drash, et al., 1990; Orchard, Dorman, Maser, Becker, Ellis, et al., 1990). Briefly, study participants were diagnosed between 1950 and 1980 and seen within 1 year of diagnosis at Children’s Hospital of Pittsburgh. Although this population is clinic based, it has been shown to be representative of the type 1 diabetes population of Allegheny County, Pennsylvania (Wagener, Sacks, LaPorte, & MacGregor, 1982). Prior to their scheduled clinic visit, participants were sent questionnaires concerning demographic, health care, self-care, and medical history information. The study design employed for this analysis was a nested case-control examining the association between serum levels of antioxidants, as well as TAR, with CAD incidence during the 10-year follow-up period using stored samples.

2.1. Case-control selection

Cases were defined as those participants who first developed CAD as determined by EDC physician-diagnosed angina or MI confirmed by Q-waves on electrocardiogram (ECG) or hospital records (Minnesota code 1.1 or 1.2), or angiographic stenosis ≥50%, coronary artery bypass surgery, angioplasty, or ischemic ECG changes (Minnesota codes 1.3, 4.1, 4.2, 4.3, 5.1, 5.2, 5.3, 7.1) during the follow-up period. Cases were pair matched by gender, age, and diabetes duration (±3 years) to controls who did not have CAD. Samples and risk factors used in analyses were identified from the earliest exam prior to incidence in the cases. Fifty-four cases and 67 controls (including 36 matched pairs) had stored samples and full covariate information available for analyses.

2.2. Definition of EDC complications and risk factors

Use of antioxidant vitamin supplements was assessed by a standardized medical history questionnaire, although information on specific supplements and dosage was not recorded. Blood pressure was measured with a random zero sphygmomanometer, according to the Hypertension Detection and Follow-Up Program protocol, after a 5-min rest (Borhani et al., 1976). Hypertension was defined as ≥140/90 mm Hg or use of antihypertensive medication. Smoking status (ever/never) was obtained via self-report. Depressive symptomatology was assessed using the Beck Depression Inventory (Beck & Garbin, 1988).

Urinary albumin was determined immunonephelometrically (Ellis & Buffone, 1977), and overt nephropathy was defined as albumin excretion rate (AER) >200 µg/min in at least two of three timed urine samples (24 h, overnight, random timed postclinic) or, in the absence of urine, a serum creatinine >2 mg/dl or, renal failure or renal transplant. Estimated glucose disposal rate (eGDR-insulin sensitivity) was estimated by a regression equation derived from hyperinsulinemic euglycemic clamp studies on 24 subjects chosen to represent the full spectrum of insulin resistance (IR) as represented by IR risk factors (Williams, Erbey, Becker, Arslanian, & Orchard, 2000). White blood cell count (WBC) was obtained using a counter S-plus IV and fibrinogen using a biuret colorimetric procedure and a clotting method.

2.3. Laboratory methods

High-density lipoprotein cholesterol was determined by a precipitation technique (heparin and manganese chloride) with a modification (Warnick & Albers, 1978) of the Lipid Research Clinics method (National Institutes of Health &
Department of Health, 1975). Cholesterol and triglycerides were measured enzymatically (Allain, Poon, Chan, Richmond, & Fu, 1974; Bucolo & David, 1973). Low-density lipoprotein cholesterol levels were calculated from measurements of the levels of total cholesterol, triglycerides, and HDL cholesterol using the Friedewald equation (Friedewald, Levy, & Fredrickson, 1972). Stable glycosylated hemoglobin (HbA1c) was measured by ion exchange chromatography (Isolab, Akron, OH) and subsequently by automated high-performance liquid chromatography (Diamat, BioRad, Hercules, CA). Readings with the two methods are almost identical ($r= .95$).

Total antioxidant reserve in serum was assayed by chemiluminescence produced in the presence of luminol and a source of peroxyl radicals, as previously described (Tyurina et al., 1995). A water-soluble azoinitiator, 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), was used to produce peroxyl radicals at a constant rate as previously described (ElSayed, Tyurina, Menshikova, Kisin, & Kagan, 1996). Oxidation of luminol (400 μM) by AAPH-derived peroxyl radicals in 50 mM disodium phosphate buffer (pH 7.4) at 37°C was assayed by monitoring chemiluminescence response. The reaction was started by the addition of AAPH. A delay in the chemiluminescence response, which is caused by interaction of endogenous antioxidants with AAPH-derived peroxyl radicals, was observed upon addition of plasma. Based on the known rate of peroxyl radical generation by AAPH, the amount of peroxyl radicals scavenged by endogenous antioxidants of plasma was evaluated. A Microlite ML 1000 microtiter plate luminometer (Dynatech Labs, Chantilly, VA, USA) was used for the determinations.

The concentration of sulfhydryl groups (low-molecular-weight thiols and protein sulfhydryl groups) in plasma samples was determined using ThioGlo-1 (Covalent Associated, Woburn, MA), a maleimide reagent that produces a highly fluorescent product upon its reaction with sulfhydryl groups (Langmuir, Yang, LeCompte, & Durand, 1996). A standard curve was established by addition of glutathione (0.04–4.0 μM) to 50 mM disodium phosphate buffer (pH 7.4) containing 10 μM ThioGlo-1. Low-molecular-weight thiols (LMWT) content was estimated by an immediate fluorescence response observed upon addition of ThioGlo-1 to plasma. Levels of total protein sulfhydryls were determined as an additional fluorescence response after addition of 4 mM SDS to the sample. A Cytofluor 2350 fluorescence plate reader (Millipore, Marlborough, MA, USA) was used to detect fluorescence using excitation and emission wavelengths of 388 and 500 nm, respectively. The data acquired were exported from the spectrophotometer using Cytofluor software. The assessment of LMWT allowed differentiation with homocysteine concentration.

For retinol, α-, and γ-tocopherol (Driskell, Neese, Bryant, & Bashor, 1982; Miller, Lorr, & Yang, 1984) all procedures were performed under subdued lighting. Standard solutions of retinol acetate (320 μg/ml) in ethanol were prepared and exact concentrations determined spectrophotometrically (absorption coefficient: 3450 at 472 nm for retinol acetate). An extractant solution was prepared containing 1 mg ascorbic acid, 10 μg retinol acetate, and 10 μl water per milliliter of ethanol.

Aliquots (500 μl) of serum were mixed with 500 μl of extractant, and the samples shaken vigorously (vortexed for 10 min) with 6 ml petroleum ether. Then the samples were centrifuged at 800×g for 5 min; the supernatants collected and dried under nitrogen. To measure retinol, α-, and γ-tocopherol, the aliquot was resuspended in 50 μl ethanol and 25 μl analyzed by HPLC. The column was a microsorb-MV C18 (5 μm; 4.6 mm×25 cm; Rainin), and the solvent was methanol flowing at 2 ml/min. The HPLC was a model 1090 (Hewlett-Packard), and the detector a UV diode array model 1040 (Hewlett-Packard). Components were monitored at 290 nm, and the calculations were based on peak areas and the relative intensities determined using mixtures of authentic standards. The coefficient of variation between runs was 4.0%, 2.8%, and 6.0% for retinol, α-tocopherol, and γ-tocopherol respectively.

### 2.4. Statistical analyses

Logarithmic transformation was performed before statistical testing for variables not following a normal distribution (i.e., calories expended in physical activity, triglycerides, AER, and plasma antioxidant concentration).

### Table 1

Baseline characteristics by CAD incidence

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Cases* (n=54)</th>
<th>Controls* (n=67)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34.2 (7.1)</td>
<td>35.0 (6.7)</td>
<td>.56</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>26.9 (7.4)</td>
<td>26.5 (7.5)</td>
<td>.77</td>
</tr>
<tr>
<td>Percent male</td>
<td>50.0 (27)</td>
<td>46.3 (31)</td>
<td>.68</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2 (3.4)</td>
<td>24.1 (3.0)</td>
<td>.86</td>
</tr>
<tr>
<td>WHR</td>
<td>0.85 (0.08)</td>
<td>0.80 (0.07)</td>
<td>.002</td>
</tr>
<tr>
<td>Percent ever smoked (n)</td>
<td>40.7 (22)</td>
<td>29.9 (20)</td>
<td>.21</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>10.8 (1.8)</td>
<td>10.2 (1.6)</td>
<td>.07</td>
</tr>
<tr>
<td>cGDR (mg/kg per minute)</td>
<td>6.8 (2.2)</td>
<td>8.1 (2.2)</td>
<td>.002</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>119.3 (16.5)</td>
<td>117.8 (18.0)</td>
<td>.63</td>
</tr>
<tr>
<td>Percent hypertension (%)</td>
<td>37.0 (20)</td>
<td>22.4 (15)</td>
<td>.08</td>
</tr>
<tr>
<td>Overt nephropathy (%)</td>
<td>31.5 (17)</td>
<td>9.0 (6)</td>
<td>.002</td>
</tr>
<tr>
<td>Calorics expended in physical activityb</td>
<td>1308.5 (1398.8)</td>
<td>2120.9 (3522.6)</td>
<td>.01</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>196.1 (40.3)</td>
<td>182.8 (30.9)</td>
<td>.05</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>124.0 (76.7)</td>
<td>74.0 (41.8)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>AER (μg/min)</td>
<td>447.4 (960.8)</td>
<td>294.2 (1188.7)</td>
<td>.0002</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>325.0 (112.9)</td>
<td>282.9 (86.4)</td>
<td>.03</td>
</tr>
<tr>
<td>WBC×10³/mm³</td>
<td>7.6 (2.2)</td>
<td>6.7 (1.9)</td>
<td>.01</td>
</tr>
<tr>
<td>Use of supplements (%)</td>
<td>27.8 (15)</td>
<td>26.9 (18)</td>
<td>.91</td>
</tr>
<tr>
<td>α-Tocopherol (μg/ml)c</td>
<td>10.0 (5.2)</td>
<td>10.9 (4.1)</td>
<td>.32</td>
</tr>
<tr>
<td>γ-Tocopherol (μg/ml)c</td>
<td>3.3 (2.1)</td>
<td>3.0 (1.8)</td>
<td>.48</td>
</tr>
<tr>
<td>Retinol (μg/ml)b</td>
<td>0.62 (0.25)</td>
<td>0.61 (0.27)</td>
<td>.66</td>
</tr>
<tr>
<td>Thiols (μmol/L)b</td>
<td>91.1 (20.8)</td>
<td>99.0 (27.0)</td>
<td>.14</td>
</tr>
<tr>
<td>TAR (μmol/L)b</td>
<td>1.5 (0.38)</td>
<td>1.4 (0.33)</td>
<td>.12</td>
</tr>
</tbody>
</table>

* Values are means (standard deviation).

b Log-transformation performed before statistical testing.

c Adjusted for cholesterol and log triglycerides.
Student’s t-tests and $\chi^2$ tests were used to examine univariate associations between risk factors and CAD incidence. Cox proportional hazard models were conducted to assess the multivariable association between serum antioxidants and CAD incidence. Time-dependent variables were constructed when the proportional hazards assumptions were not satisfied (i.e., time-dependent variables were created for WBC, and the log of triglycerides). Due to the small sample size, we did not have adequate power to perform paired analyses, and we conducted unpaired analyses, including matching variables in all models (with the exception of the variable indicating the participants’ age, because it was highly correlated with diabetes duration). Since tocopherols are carried in lipoprotein particles, individuals with higher concentrations of lipids appear to have higher tocopherol concentration (Hunter, 1998). Thus, adjustment for total cholesterol and triglycerides was performed in all analyses involving $\alpha$- and $\gamma$-tocopherol. Effect modification by antioxidant vitamin use was examined. Separate models were conducted first allowing for serum antioxidant vitamins, then replacing antioxidants with TAR. Analyses were conducted using SAS version 8.0 (SAS Institute, Cary, NC). Hazard rates for continuous variables are reported per 1 S.D. increase. Variables were considered significant predictors at $P<.05$.

The University of Pittsburgh Institutional Board approved the study protocol.

### 3. Results

At baseline, study participants who subsequently developed CAD were more likely to have higher mean WHR, triglycerides, fibrinogen, WBC count, and lower eGFR, and were more likely to have been diagnosed with overt nephropathy. Controls appeared more physically active, as shown by the greater amount of calories expended in physical activity (Table 1). No differences were observed in the concentration of serum antioxidants or TAR between incident CAD cases and controls.

**Table 2**

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>All subjects (n=121; 54 cases, 67 controls)</th>
<th>Nonusers (n=88; 39 cases)</th>
<th>Users (n=33; 15 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Base model</strong></td>
<td>Not selected</td>
<td>Not selected</td>
<td>Not selected</td>
</tr>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log kilocalorie expended in physical activity</td>
<td>2.57 (1.36–4.87)</td>
<td>Not selected</td>
<td>3.62 (1.68–7.83)</td>
</tr>
<tr>
<td>Overt nephropathy</td>
<td>1.42 (1.07–1.88)</td>
<td>Not selected</td>
<td>1.40 (1.02–1.94)</td>
</tr>
<tr>
<td>WBC $\times 10^3$/mm$^2$</td>
<td>1.61 (1.30–2.00)</td>
<td>1.81 (1.42–2.31)</td>
<td>2.02 (1.51–2.69)</td>
</tr>
<tr>
<td>Log triglycerides (mg/dl)</td>
<td>Not included</td>
<td>0.71 (0.53–0.94)</td>
<td>Not selected</td>
</tr>
<tr>
<td>Log $\alpha$-tocopherol (µg/ml)</td>
<td>Not included</td>
<td>Not included</td>
<td>276.25</td>
</tr>
<tr>
<td>Antioxidant use</td>
<td>Not included</td>
<td>Not included</td>
<td>62.55</td>
</tr>
<tr>
<td>AIC</td>
<td>418.19</td>
<td>414.71</td>
<td></td>
</tr>
</tbody>
</table>

Values are HR (95% CI).

AIC: Akaike’s Information Criterion.

* The base model also included diabetes duration, gender, smoking status, HbA1c, hypertension, fibrinogen, total cholesterol, log $\gamma$-tocopherol, log retinol, and log thiol.

In Cox proportional hazards models with backward elimination (Table 2), the base model included diabetes duration, gender, smoking status, calories expended in physical activity, HbA1c, hypertension, overt nephropathy status, fibrinogen, WBC count, total cholesterol, and triglycerides. Overt nephropathy status, WBC count, and triglycerides emerged as the only significant predictors of CAD incidence. Adding serum antioxidants, we found that $\alpha$-tocopherol was also a significant predictor of CAD incidence. However, when checking for effect modification by use of antioxidant supplements, a significant interaction was observed with $\alpha$-tocopherol concentration ($P=.0004$).

Running separate models for users and nonusers of antioxidant vitamin supplements, we observed a significant effect of $\alpha$-tocopherol only among supplement users.

Creating tertiles of serum $\alpha$-tocopherol concentration (Fig. 1), we found that the rates of CAD among users of antioxidant vitamin supplements were higher in the first two tertiles, but much lower in the third tertile, compared to nonusers. There was a significant decreasing trend among users of antioxidants ($P=.02$), whereas among nonusers...
there was a slight elevation in risk from the first to the third tertile (not significant).

To address the question of whether the observed effect was primarily due to antioxidant supplementation or other factors associated with supplement use, we first looked at differences between users and nonusers. We observed greater rates of hypertension as well as a higher average systolic blood pressure among users of antioxidant vitamin supplements (Table 3), who were also more likely to have been diagnosed with overt nephropathy. As expected, supplement users also had higher concentrations of \( \alpha \)-tocopherol and retinol.

\( \alpha \)-Tocopherol levels were then compared between cases and controls after stratification by antioxidant vitamin supplement use or renal disease. Among those using antioxidant vitamins and among those with renal disease, controls had higher levels of \( \alpha \)-tocopherol than cases, the effect being a little stronger for the presence of renal disease. A greater concentration of \( \alpha \)-tocopherol was also observed in controls among those with serum creatinine levels indicating abnormal renal function (creatinine concentration \( \geq 1.4 \) and \( \geq 1.5 \) mg/dl for female and male participants, respectively) (Table 4).

We therefore checked for effect modification by either overt nephropathy status or antioxidant vitamin use. Both interaction terms were significant (\( P=.02 \) for the interaction with overt nephropathy, \( P=.0004 \) for the interaction with antioxidant use), although, when allowing for both interaction terms in the same model, only the interaction term between \( \alpha \)-tocopherol and antioxidant use was selected. Nevertheless, running separate analyses for individuals with and without renal disease, we observed a protective effect of \( \alpha \)-tocopherol only in those with renal disease (HR=0.46, 95% CI=0.23–0.91).

Surprisingly, in analyses replacing serum antioxidants with TAR, the latter was positively associated with CAD incidence (HR=1.40, 95% CI=1.06–1.85); no interaction was observed between serum TAR and either antioxidant use or renal disease (not shown).

### 4. Discussion

Our study results suggest an inverse association between serum \( \alpha \)-tocopherol concentration and CAD incidence only among users of antioxidant supplements or individuals with renal disease in type 1 diabetes mellitus. No effect was observed among nonusers of antioxidant vitamins, even for the same concentration of \( \alpha \)-tocopherol. Other antioxidant vitamins generally did not significantly improve the prediction of CAD, and TAR was, surprisingly, positively associated with CAD incidence.

The potential of antioxidant vitamins to provide protection against the development of several chronic disorders and cardiovascular disease remains controversial. Possible biological mechanisms of antioxidant action include the protection of LDL against oxidation to reduce its atherogenicity, despite the lack of evidence linking decreased LDL oxidation to decreases in atherosclerosis (Diaz et al., 1997). Additional mechanisms may include inhibition of monocyte adhesion and platelet activation, protection against cytotoxic effects of oxidized LDL, and preservation of endothelium-derived nitric oxide activity (Diaz et al., 1997).

Interestingly, in the EDC study population, individuals who reported use of antioxidant vitamin supplements were of poorer health and at higher risk of cardiovascular disease, as shown by the higher proportions suffering from hypertension and nephropathy, than participants who did not use antioxidant supplements. As information on both disease...
status and use of antioxidant vitamins was established at baseline, we could not investigate whether patients self-medicated using dietary supplements, or whether supplement use preceded disease. However, a number of published reports suggest that use of dietary supplements is more common among individuals with medical conditions than among healthy participants (Bender, Levy, Schucker, & Yetley, 1992; Houston, Johnson, Daniel, & Poon, 1997; Kato, Nomura, Stehmann, & Chyou, 1992; Lyle, Mares-Perlman, Klein, Klein, & Greger, 1998; Martin, 1998; Satia-Abouta et al., 2003; White-O’Connor, Sobal, & Muncie, 1989).

In the high-risk group of participants with overt nephropathy and among antioxidant vitamin users, increases in the serum concentration of α-tocopherol were associated with a significant decreased risk of CAD incidence, independent of traditional cardiovascular disease risk factors, or other serum antioxidants. Because the majority (79%) of participants who used antioxidant supplements took multivitamin pills, it is possible that the observed effect of α-tocopherol on CAD is attributable to another vitamin commonly included in multivitamin preparations, or even a combination of vitamins. However, adjusting for measured serum antioxidants and/or TAR did not alter the effect of α-tocopherol.

This potential protective effect of α-tocopherol among patients with increased albumin excretion may suggest that individuals at higher risk are the ones benefiting more from the effect of dietary antioxidants. In the Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease (SPACE) trial (Boaz et al., 2000), daily administration of 800 IU of vitamin E reduced the risk of cardiovascular endpoints by 54% among hemodialysis patients with a history of cardiovascular disease. Salonen et al. (2003) also noted trends toward a greater effect for smokers, those with lower baseline plasma vitamin C levels, or individuals who already had common carotid artery plaques at baseline, providing further support that antioxidants may help more high-risk populations. In the present study, we did not assess serum levels of ascorbic acid, because unlike tocopherols or retinol, it deteriorates rapidly during frozen storage (Comstock, Norkus, Hoffman, Xu, & Helzlsouer, 1995).

We observed a direct association between TAR and CAD incidence. This is not surprising, since total antioxidant status represents a mixture of mutually compensated terms. Indeed, it has been previously shown among Finnish men that the strongest determinants of plasma antioxidative capacity and serum lipid resistance to oxidation were ascorbic acid and urate (Nyysonen, Porkkala-Sarataho, Kaikkonen, & Salonen, 1997). Because uric acid levels are often elevated among individuals with diabetes complications (Diaz et al., 1997; Freedman, Williamson, Gunter, & Byers, 1995; Levine, Dyer, Shekelle, Schoenberger, & Stamler, 1989), it is likely that TAR reflects this trend and shades depletion of other antioxidants (such as vitamins C and E) that may occur on a relatively smaller scale. Thus, the total antioxidant status may not be as informative as its integral components. Previously published reports have produced conflicting results for an association between total antioxidant status and cardiovascular outcomes (Nieto, Iribarren, Gross, Comstock, & Cutler, 2000; Nojiri et al., 2001; Valabhji et al., 2001; Woo et al., 1997).

There were several limitations in this study. First, the concentration of serum antioxidants was assessed at one point in time but was not reevaluated for the duration of the follow-up. Thus, although serum tocopherols and retinol reflect long-term intake and it would be unlikely that they would change with respect to dietary changes, changes in supplementation habits would be of concern. Information on the supplementation dose was not available either, and, thus, dose–response could not be examined. Other potential limitations include the small sample size and the storage of samples for up to 10 years before analyses. However, it is unlikely that storage would differentially affect cases and control subjects since storage time was similar between the two groups.

None of the above limitations allows for definite conclusions, and we can only speculate that perhaps the particularly high levels of α-tocopherol seen among those with renal disease may provide some protection against CAD incidence, and, as also suggested by previous research studies (Boaz et al., 2000; Salonen et al., 2003), population subgroups at higher risk are the ones benefiting from antioxidants. Conversely, an unidentified factor unique to supplement users may be responsible for the observed effect of α-tocopherol.

References


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