Vitamin E and Coronary Heart Disease in Tunisians

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Background: Vitamin E (VE) is thought to be effective in preventing atherosclerosis. However, to date no consistent relationship has been identified between VE and coronary heart disease (CHD). This study was designed to assess the degree of association between VE and CHD in a sample of the Tunisian population.

Methods: Sixty-two angiographically confirmed coronary atherosclerotic patients and 65 age- and sex-matched controls were included. VE was measured in plasma and in the LDL fraction by HPLC. Cholesterol, triglycerides, and phospholipids were measured by enzymatic methods.

Results: A trend toward a meaningful decrease of plasma VE was observed in affected patients compared with controls (P = 0.06). VE concentrations standardized for cholesterol and lipid concentrations were significantly lower (P <0.02) in coronary patients than in controls (4.35 ± 1.03 vs 4.82 ± 1.23 mmol/mol for cholesterol-adjusted VE and 2.35 ± 0.56 vs 2.66 ± 0.65 mmol/mol for lipid-adjusted VE, respectively). In the LDL fraction, only cholesterol-standardized VE was significantly lower in cases than controls (3.84 ± 1.13 vs 4.41 ± 1.16 mmol/mol). This association between VE and CHD remained unchanged independent of age, sex, smoking habit, hypertension, and diabetes. In CHD patients, lower lipid-adjusted VE was associated with enhanced LDL susceptibility to oxidation but without alteration of the serum fatty acid profile.

Conclusions: These results support the hypothesis that VE plays a role in preventing atherosclerosis. © 2000 American Association for Clinical Chemistry

Vitamin E (VE),5 the main lipid-soluble antioxidant, is thought to contribute to the prevention of atherosclerosis through inhibition of oxidation of LDL (1–3). Previous studies that looked into the association of VE and coronary heart disease (CHD) did not show an unequivocal relationship between them. A negative correlation had been reported between serum VE mean concentrations and coronary mortality rates in several European populations, suggesting a protective effect of VE against atherosclerosis (4). A negative association was also observed between VE and CHD morbidity and mortality in studies based on blood measurement, dietary intake, or supplementation (5–8). In contrast, no consistent relationship was found between VE and myocardial infarction or CHD death risks in other epidemiological prospective studies (9–12).

Taking into account the influence on the atherogenic process of the balance between antioxidants and oxidizable substrates, the purpose of the present case-control study was to assess the association between lipid-standardized VE and CHD in a sample of the Tunisian population. Because of the antioxidant effect of VE, we tested in vitro LDL susceptibility to oxidation and analyzed serum fatty acid profiles.

Subjects and Methods

STUDY POPULATION
Cases. We studied 62 patients with angiographically confirmed coronary atherosclerosis (50 males and 12 fe-
males), ages 35–80 years (mean, 58 years), between January and June 1997. All were seen by the Cardiology Exploration Service of Rabta Hospital (Tunis).

Controls. Sixty-five individuals without a past record of clinical coronary manifestations (49 males and 16 females), ages 32–85 years (mean, 56 years), were selected by the Biochemistry Laboratory of Rabta Hospital as part of a systematic blood examination in the same period.

Both groups were investigated with full informed consent. They were questioned about their smoking and eating habits, drug consumption, and major risk factors of CHD (hypertension, diabetes, and dyslipidemia). Dyslipemic subjects [cholesterol ≥6.5 mmol/L and/or triglycerides (TGs) ≥2.3 mmol/L] as well as individuals receiving lipid-lowering drugs were not included. Diabetic and hypertensive subjects were adhering to the necessary diet and drugs; the other subjects consumed a regular Tunisian diet [rich in carbohydrates, vegetables, and fruit with moderate fat and proteins intake (13)]. No subjects were taking vitamin supplements. Subjects’ weight in kilograms (W) and height in meters (H) were measured, and body mass index (BMI) was calculated using the formula: BMI = W/H².

Fasting blood samples were collected into EDTA-containing tubes and immediately centrifuged at 1500 × g for 10 min. Plasma was stored at −20 °C, away from light, until VE analysis (within 4 weeks) or at −80 °C, supplemented with butylated hydroxy-toluene (BHT), for fatty acid analysis. An aliquot of each serum sample was kept at 4 °C for lipid assays and LDL oxidative susceptibility testing (within 24 h). Serum or plasma for fatty acid analysis and susceptibility of LDL to oxidation assay was collected from 54 randomly chosen subjects.

ANALYTICAL METHODS
VE was assessed by HPLC as described by Driskell et al. (14). Briefly, plasma was deproteinized in the presence of ethanol-BHT containing retinol acetate as internal standard. VE was extracted using hexane, and then evaporated to dryness under a stream of nitrogen. The residues were redissolved in methanol-BHT and injected into the column. The HPLC system consisted of a 20-μL injection loop (7125; Rhodyne), a bio-liquid pump (LC-7A), and a C₁₈ reversed-phase column [CLC ODS (M) 25], an ultraviolet-visible spectrophotometric detector (SPD-7AV), and an electronic integrator (C-R6A; all from Shimadzu). The mobile phase consisted of methanol (gradient grade; Merck) at a flow rate of 1.5 mL/min. The within-day (n = 20) and long-term (n = 30) imprecision (CVs) was 4.2% and 4.5%, respectively, at a concentration of 23 μmol/L.

LDL was precipitated with heparin, and then isolated (Randox). HDL was isolated after precipitation of LDL and VLDL by phosphotungstate-MgCl₂ reagent (15). Total cholesterol (TC), TGs, and phospholipids (PLs) in serum and in precipitated fractions were analyzed by enzymatic methods (Biomagreb). Because of the strong correlation between VE and lipids (r = 0.40; P < 0.001), results of VE were also expressed as the ratios VE/TC and VE/TLs where TLs = TC + TGs + PLs.

Susceptibility of LDL to oxidation was tested by formation of thiobarbituric acid reacting substances (TBARS) after phenylhydrazine exposure (16), and serum fatty acid profiles were determined by capillary gas chromatography (17).

STATISTICAL ANALYSIS
Values were reported as the mean ± SD and as a percentage for quantitative and categorical variables, respectively. The χ² test was used for associations between categorical variables, and the t-test was used for means comparisons. To assess how the association between CHD and VE depended on sex, age, smoking, hypertension, and diabetes, multivariate logistic regression was applied. Computations were performed by SAS statistical package (SAS Institute). Goodness-of-fit of logistic models were satisfactory.

Results

DEMOGRAPHIC AND ANTHROPOMETRIC CHARACTERISTICS AND LIPID VALUES
Hypertension and diabetes were significantly more common in CHD patients than controls. HDL-cholesterol was lower and TGs were higher (Table 1). No statistically significant differences were observed between CHD patients and control individuals in sex distribution, age, BMI, smoking, or mean serum TC and PLs (Table 1).

VE AND LIPID-STANDARDIZED VE IN SERUM AND LDL
An apparently lower serum VE in CHD patients than in controls was not statistically significant (P = 0.06). The ratios VE/TC and VE/TLs were significantly lower among patients. In the LDL fraction, VE concentrations and VE/TLs were similar in the two groups, whereas the

| Table 1. Comparison between CHD patients and controls according to demographic and anthropometric characteristics, CHD risk factors, and lipid concentrations. a |
|-----------------|-----------------|------|
|                  | Patients (n = 62) | Controls (n = 65) | P      |
| Men, %           | 80.6            | 75.4 | NS b   |
| Age, years       | 58.2 ± 9.2      | 56.4 ± 13.1 | NS     |
| BMI, kg/m²       | 26.2 ± 3.2      | 25.8 ± 3.1 | NS     |
| Smoking, %       | 54.8            | 52.3 | NS     |
| Hypertension, %  | 43.5            | 12.3 | <10⁻⁴  |
| Diabetes, %      | 38.7            | 6.15 | <10⁻⁶  |
| TGs, mmol/L      | 1.52 ± 0.65     | 1.26 ± 0.39 | <10⁻²  |
| HDL-cholesterol, mmol/L | 1.01 ± 0.36 | 1.19 ± 0.28 | <10⁻²  |
| TC, mmol/L       | 4.57 ± 0.89     | 4.55 ± 0.77 | NS     |
| PLs, mmol/L      | 2.36 ± 0.48     | 2.38 ± 0.49 | NS     |

a Age, BMI, and lipid values expressed as means ± SD; other results expressed as percentages.

b NS, not significant.
VE/TC ratio was significantly lower in CHD patients (Table 2). Analysis after adjustment for age, sex, smoking, hypertension, and diabetes showed a significant association between CHD and VE ($P = 0.04$), the VE/TC ratio ($P = 0.02$), and the VE/TLs ratio ($P = 0.03$).

**Susceptibility of LDL to Oxidation and Fatty Acid Profile**

The susceptibility of LDL to oxidation and the fatty acid profile were tested in 35 CHD patients and 19 controls. In this subgroup, cases and controls were comparable in age, sex-ratio, smoking, BMI, TC, and PLs. Hypertension and diabetes were more common in cases. TGs were higher and HDL-cholesterol was lower in CHD patients than controls. The susceptibility of LDL to oxidation was significantly higher among affected patients than controls. On the other hand, the serum fatty acid profile was not significantly different between the two groups (Table 3). No significant correlation was observed between lipid-adjusted VE and TBARS formation or percentages of saturated, monounsaturated, or polyunsaturated fatty acids.

**Discussion**

This study revealed a significant decrease of VE adjusted for cholesterol and lipids among CHD patients. This decrease is not affected by potential confounding factors (sex, age, smoking, hypertension, and diabetes). These findings corroborate the hypothesis that VE has a protective effect against atherosclerosis. Our results are consistent with the significantly lower VE concentrations observed in patients with CHD (8, 18, 19). They are also consistent with the inverse correlation reported between VE and ischemic heart diseases mortality (4) as well as the risk of angina pectoris (5). On the basis of VE consumption, two well-controlled prospective cohort studies, including respectively, 87145 and 39910 individuals, showed that a daily intake of 100 IU or more of VE from supplements for a minimum of 2 years decreases CHD morbidity and mortality (6, 7). This relationship has persisted after adjustment for many potential confounding factors. Other large-scale observational studies have provided evidence of an association between high intake of VE and lower risk of CHD (20–23). In contrast, some studies failed to detect an association between serum VE concentration or VE consumption and subsequent myocardial infarction or CHD death (9–12, 24–26). The lack of association in these studies may be attributable to methodological issues such as inaccuracies in the determination of intake, changes in dietary habits during the follow-up period, or a decrease in VE concentrations with time in archived serum (9, 10). The insufficient variation of dietary VE intake (26) or serum VE concentration within the study population (9, 25) may have also weakened this association. Differences in the selection criteria of cases (e.g., questionnaire, medical examination, and angiography) and in VE status determination (e.g., dietary intake, vitamin supplementation, and lipid-standardized or absolute plasma VE concentrations) may have modified the relationship between VE and CHD. In our study, all cases were angiographically confirmed and VE was related to cholesterol and TLs. Indeed, lipid-adjusted VE is a better indicator of VE status than absolute plasma VE (27–30). The VE/TC ratio of 4.82 observed in our control sample supports the tentatively suggested borderline of VE adequacy of 4.80–5.00 (30, 31).

Unlike the mostly observational cohort studies that support a beneficial effect of VE in CHD, controlled trials that tested this hypothesis in populations with different cardiovascular risk backgrounds did not show a clear role of VE in preventing atherosclerosis (32–36). The lack of benefit of supplementation could be related to several factors, such as the low doses used (32, 33), the small numbers of events (34), the limited period of treatment (34, 35), the advanced age and high CHD risk of participants (33, 35, 36), and the use of clinical events as endpoints (32–36). Because VE mainly prevents the initiation of lesions, supplementation of high-CHD risk older individuals (who probably had constituted atherosclerotic lesions) for a limited period (≤5 years) may have no evident effect, especially when evaluated on the basis of clinical events. However, supplementation of younger and CHD-free subjects for prolonged periods (>10 years), using imaging criteria as endpoints, could confirm the suggested protective effect of VE in CHD.

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**Table 2. VE and lipid-standardized VE in serum and LDL in CHD patients and healthy controls.**

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 62)</th>
<th>Controls (n = 68)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VE, μmol/L</td>
<td>19.90 ± 5.83</td>
<td>21.69 ± 5.24</td>
<td>0.06</td>
</tr>
<tr>
<td>VE/TC, mmol/mol</td>
<td>4.35 ± 1.03</td>
<td>4.82 ± 1.23</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>VE/TLs, mmol/mol</td>
<td>2.35 ± 0.56</td>
<td>2.66 ± 0.65</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVE, μmol/L</td>
<td>9.50 ± 3.27</td>
<td>10.27 ± 3.32</td>
<td>NS</td>
</tr>
<tr>
<td>LVE/LTC, mmol/mol</td>
<td>3.84 ± 1.13</td>
<td>4.41 ± 1.16</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LVE/TLs, mmol/mol</td>
<td>2.61 ± 0.58</td>
<td>2.69 ± 0.55</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Results are expressed as means ± SD.

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**Table 3. TBARS formation and fatty acid profile in CHD patients and controls.**

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 35)</th>
<th>Controls (n = 19)</th>
<th>P</th>
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<tbody>
<tr>
<td>TBARS, μmol/L</td>
<td>96 ± 29</td>
<td>83 ± 29</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SFA, weight %</td>
<td>30.7 ± 3.0</td>
<td>29.4 ± 2.0</td>
<td>NS</td>
</tr>
<tr>
<td>MUFA, weight %</td>
<td>23.4 ± 4.5</td>
<td>23.8 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>PUFA, weight %</td>
<td>46.4 ± 6.1</td>
<td>47.1 ± 4.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

* SFA, saturated fatty acids; NS, not significant; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
The decrease in VE concentrations observed in our CHD patients could be of either nutritional or metabolic origin. Because VE has an exclusively dietary origin, any restricted intake may cause deficiency. On the other hand, increased consumption as part of antioxidative reaction may also explain the decrease. It is not clear whether the VE decrease is a result of the disease or contributes to its further aggravation. However, the strong association observed between low VE concentrations or intake and high risk of CHD events (4–7, 20–23) suggests that VE inadequacy could be a risk factor of coronary disease.

The protective effect of VE against atherogenesis could be attributable to its demonstrated role in inhibiting LDL oxidation. An improved antioxidative resistance of LDL has not only been shown for high supplementation of VE (1–3, 37–40), but it starts to become significant at relatively low VE intake compared with VE-rich diets (41). Oxidized LDLs are atherogenic through their enhanced uptake by unregulated macrophage scavenger receptor, their immunogenicity, and their induction of chemotactic, cytotoxic, and growth factors (42–45). The absence of association between lipid-adjusted VE and both LDL oxidizability and the fatty acid profile in our CHD patients cannot be interpreted as evidence against the antioxidative role of VE, but rather as evidence that oxidative resistance also depends on many other antioxidant factors. In addition to its antioxidant function in LDL, VE has the potential to prevent other deleterious effects involved in the pathogenesis of atherosclerosis. VE decreases interleukin I secretion and monocyte endothelial cell adhesion (46). It also reduces platelet adhesion and aggregation (47) as well as cultured smooth muscle cell proliferation (48). VE inhibits vitamin K-dependent clotting factors (49), prevents activation of interleukin I gene expression (50), and prevents enhanced collagen synthesis by decreasing procollagen I gene transcription in fibroblasts (51). VE also modulates synthesis of prostanoids and other host defenses, which are important for the immune response (52).

In conclusion, the serum VE concentration was lower in coronary patients than in unaffected controls. The decrease was significant only when VE was expressed in relation to cholesterol or TLs. This decrease was found to be associated with an increase of LDL oxidizability but not with an alteration in serum fatty acid profile. VE could be one of the several factors giving a degree of protection against atherosclerosis. Therefore, adequate VE ingestion from diet or supplements could prevent CHD.

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