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An Investigation of the Relationship between Serum Vitamin E Status and Coronary Risk Factors in Dyslipidaemic Patients

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Abstract

Objective
Vitamin E is a major lipid-soluble antioxidant. It has been demonstrated that vitamin E supplementation has a beneficial effect against coronary heart disease (CHD). This study to investigate the effects of coronary risk factors on serum vitamin E status in patients with dyslipidaemia.

Materials and Methods
This tertiary care hospital; case - control study was comprised 237 dyslipidaemic patients and 135 healthy individuals recruited from university and hospital employees. Serum vitamin E concentration was measured using high performance liquid chromatography (HPLC).

Results
Compared to the healthy individuals, the dyslipidaemic patients had higher serum vitamin E ($p<0.001$), but serum vitamin E / total cholesterol ratio did not differ between patients and healthy controls ($p>0.05$). Serum vitamin E did not differ between subcategories of dyslipidaemic patients with and without coronary risk factors ($p>0.05$), but Serum vitamin E / total cholesterol ratio was higher in patients with established coronary heart disease ($p<0.01$), hypertriglyceridaemia ($p<0.05$) and metabolic syndrome ($p<0.05$). In the patients there was not a strong association between serum vitamin E or serum vitamin E / total cholesterol ratio and coronary risk factors except for the serum cholesterol.

Conclusion
Serum vitamin E or vitamin E / total cholesterol ratio is not associated with the risk of coronary heart disease. Therefore, higher serum vitamin E or vitamin E / total cholesterol ratio doesn't seem to have a preventive role against coronary heart disease.

Keywords: Cholesterol, Coronary heart disease, Dyslipidaemia, Metabolic syndrome, Multiple regression analysis, Vitamin E

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Introduction

Vitamin E is a major lipid-soluble antioxidant in cellular membranes. It has been demonstrated that supplementation with vitamin E can inhibit the oxidation of LDL (1-3). At least part of the beneficial effects of vitamin E against coronary heart disease (CHD) may be due to decreased platelet ability to aggregate in humans (2, 4). Vitamin E may also have a beneficial acute effect on vascular endothelial function (5).

Most of the more recent work in animal models has supported the hypothesis that vitamin E supplementation can prevent or slow the development of atherosclerosis, although the results of early animal experiments were equivocal (6-9). Additionally, recent clinical studies suggest that vitamin E is also ineffectual in the primary prevention of atherosclerosis (10).

Vitamin E has also been projected to be the most effective antioxidant for lipid peroxidation (11). Epidemiological studies have shown that vitamin E is the strongest contributor to the inverse relationship between serum antioxidant concentration and IHD (12-14). In SPACE (secondary prevention with antioxidants of cardiovascular disease in end stage renal disease) trial, treatment of the patients with supplemental vitamin E was associated with a significant protective effect against cardiovascular death and non fatal MI (15). While these studies are encouraging, other studies involving patients with existing heart conditions have surprised the investigators by failing to show a benefit of vitamin E supplementation (8, 16-19). On the other hand, the results of several prospective clinical trials in human are inconsistent for the cardio protective effects of vitamin E (20-24) and the usefulness of vitamin E as a dietary supplement or adjunct therapy may be continued to be debated (25-27).

We have investigated the association between conditions related to coronary risk and serum vitamin E status.

Materials and Methods

Subjects

Two hundred and thirty-seven patients (ages 18-64 years) were recruited from the Lipid clinics at the Royal Surrey County Hospital. Each patient gave written informed concept to participate in the study. The study protocol was approved by the south- west Surrey Research Ethics Committee and the Advisory Committee of Surrey University. No specific exclusion criteria were applied, except for the patients who were already taking vitamin E supplementation. These patients were chosen because they had a high frequency of traditional coronary risk factors including obesity (35%), type 2 diabetes (18%), hypertension (79%) and positive smoking habit (18%) in the patient group, which are typical of a Lipid Clinic population.

One hundred and eighty-nine age- and sex-matched healthy controls were recruited from employees at the University of Surrey and the Royal Surrey County Hospital, Guildford. These subjects were chosen because they lived in the same area of the patients, and also they did not have any CHD, diabetes mellitus, and hypertension. Of these control subjects, 33 were obese, 9 had metabolic syndrome, and 12 were on medication. These 54 controls were excluded from the analysis comparing patients and controls. The characteristics of the patients and controls are presented in Table 1.

Blood Sampling

Blood samples were collected between 8.30 and 10.30 a.m. after a 12-h fast by venepuncture of the antecubital vein. Blood was collected into plain Vacutainer tubes (Becton-Dickenson, Cowley, Oxford, UK), allowed to clot and then serum removed.

Materials

All chemicals were obtained from Sigma (Sigma Chemical Co, Dorset, UK) unless stated otherwise.

Physical activity level

Physical activity was assessed using the James and Schofield human energy requirements equations (28).

Lipid Profiles and Blood Glucose

A full, fasted lipid profile, comprising total cholesterol, triglycerides, and high density lipoprotein (HDL) cholesterol, was determined for each patient. LDL cholesterol was calculated using the Friedwald equation (29), except for patients with triglycerides >4.0 mmol/l. Lipid and blood glucose measurements were made by routine enzymatic methods using a Bayer Advia 1650 analyzer (Bayer, Newbury, UK).

Table 1. Clinical characteristic of patients and controls.

	n (%)
Patients	237
Obese*	82 (35%)
Diabetes type 2	42 (18%)
Duration of treatment for diabetes (months)	13 (0-51)
Established coronary heart disease	55 (23%)
Unstable angina	9 (4%)
Myocardial Infarction (MI)	15 (6%)
Coronary Artery Bypass Graft (CABG)	10 (4%)
Angioplasty	13 (6%)
Angioplasty or CABG after MI	8 (3%)
Hypertension	186 (79%)
High BP*	76 (32%)
Duration of treatment for hypertension (months)	7.5 (0-72)
Moderate BP*	110 (46%)
Duration of treatment for hypertension (months)	0.0 (0-46)
Hypertriglyceridaemia*	176 (74%)
Hypercholesterolaemia*	216 (92%)
Duration of statin therapy (months)	9.0 (0-43)
Duration of fibrate therapy (months)	0.0 (0-16)
Calculated 10-year coronary risk >30%*	42 (18%)
Calculated 10-year coronary risk between 20 and 30%	54 (23%)
Metabolic syndrome*	142 (60%)
Controls	189
Normal subjects	135 (71%)
Excluded subjects	54 (29%)
Obese	33 (18%)
Metabolic syndrome	9 (4%)
Taking medications	12 (5%)

Values are expressed as median and interquartile range for duration of treatment. *Obese: Body mass index >30. *High BP: SBP>160 mmHg or DBP>100 mmHg. *Moderate BP: SBP= 130-160 mmHg or DBP= 85-100 mmHg. *Hypertriglyceridaemia: Serum triglycerides >1.7 mmol/L. *Hypercholesterolaemia: Serum total cholesterol > 5.2 mmol/L. *Calculated 10-year coronary risk: Calculated using the PROCAM algorithm (39). *Metabolic syndrome: as defined using NCEP-ATP III criteria (40).

Serum vitamin E

Serum α-tocopherol was determined by HPLC (30). Briefly, 200 µL internal standard (10 µg/ml δ-tocopherol in isopropyl alcohol) was added to 200 µL serum and vortex mixed. Aqueous ammonium sulphate (3.9 M) was added (200 µL) and the solution was again vortex mixed. After centrifugation (1000 g for 5 minutes), 50 µL of supernatant was used for analysis using a Prodigy 50 µm ODS2 (50 × 4.6 mm) column (Phenomenex Ltd, Macclesfield, Cheshire, UK) with methanol as mobile phase, and UV-detection at 294 nm. At a flow rate of 1.0 ml/minute, the retention time

for internal standard and Vitamin E were 6.6 and 8.7 minutes respectively. Vitamin E standard, internal standard (δ-tocopherol) and quality control material were obtained from BioRad Laboratories Ltd, Hemel Hempstead, UK.

Statistical analysis

Statistical analyses were carried out using the statistical package Minitab Release 13 (Minitab Inc., 3081 Enterprise Drive, State College, PA16081- 3008, USA).

Quantitative data were assessed for normality using the Kolmogorov-Smirnov tests. Normally distributed data were analysed using one-way analysis of variance. Non-normally distributed data were analysed using the non-parametric Kruskal-Wallis test. A two-sided p value of <0.05 was considered statistically significant.

Values were expressed as mean and standard error of mean (SEM) or, in the case of non-normally distributed data as median and inter-quartile range (IQR). Categorical data (such as smoking habit) were analyses using Fisher's exact or chi-square tests.

Multiple regressions were carried out to investigate the relationship between the vitamin E and possible coronary risk factors and individual components of the metabolic syndrome. Stepwise regression was used with vitamin E concentration, in turn as dependent variables and a set of independent variables.

Results

Physiological variation in vitamin E status

Gender

Serum vitamin E concentrations did not differ significantly between healthy males (n=95, 14.06±0.44 μg/mL) and females (n=94, 13.69±0.44 μg/mL) (p>0.05). Nor did serum vitamin E/ total cholesterol ratio differ significantly between healthy male (2.63±0.07 μg/mL) and female (2.50±0.07 μg/mL) subjects (p>0.05).

Age

The healthy male and female subjects were divided into 6 age categories. The age distributions for the groups were as follows: 20-29 years (n = 13 males and 13 females), 30-39 years (n = 9 males and 9 females), 40-49 years (n = 24 males and 23 females), 50-59 years (n = 28 males and 31 females), 60-69 years (n = 14 males and 11 females), and 70 years and above (n = 7 males and 7 females).

Within the male and female healthy groups which were divided into 6 groups based on their age, serum concentrations of vitamin E and vitamin E/total cholesterol ratio did not differ significantly with age (p>0.05).

Physical activity levels (PAL)

In healthy subjects, physical activity levels were not correlated with serum vitamin E concentrations (r= -0.10, p>0.05) and serum vitamin E/ total cholesterol ratio (r= -0.16, p>0.05). Nor were physical activity levels correlated with serum vitamin E concentrations (r= -0.11, p>0.05) or serum vitamin E/ total cholesterol ratio (r= -0.07, p>0.05) in the dyslipidaemic patients.

Association between dietary vitamin E and its serum level

A significant association between dietary vitamin E and its serum levels were observed in patients (r= 0.16, p=0.03) and controls (r= 0.19, p=0.02).

Pathophysiological conditions and their effects on vitamin E status

Smoking

Serum concentrations of vitamin E did not differ significantly between smokers, who had at least a 10 pack-year smoking history, (n= 66, 14.25±0.48 μg/mL) and non-smokers (n=123, 13.68±0.40 μg/mL, p>0.05). Nor did serum vitamin E/total cholesterol ratio differ significantly between smokers (2.58±0.07 μg/mL) and non-smokers (2.56±0.07 μg/mL, p>0.05).

Obesity

Serum vitamin E concentrations and vitamin E/ total cholesterol ratio did not differ significantly between obese (n=33, 13.72±0.66 μg/mL), overweight (n=58, 14.89±0.59 μg/mL), and subjects of normal weight (n=78,

13.54±0.48 μg/mL, p>0.05). Nor did serum vitamin E/total cholesterol ratio differ significantly between obese (2.45±0.10 μg/mL), overweight 2.65±0.08 μg/mL), and subjects of normal weight (2.53±0.09 μg/mL, p>0.05).

Vitamin E and CHD risk factors

Dyslipidaemic patients had significantly higher serum vitamin E concentrations in males (p<0.01) and females (p<0.01) and in the combined group (p<0.001) compared with controls. However, serum vitamin E/ total cholesterol ratio did not differ between patients and controls in males, females and the combined group (p>0.05, Table 2).

Table 2 Comparison of serum vitamin E concentrations between dyslipidaemic patients and controls.

	Patients	**Controls**
Males		
Number of subjects	142	67
Serum vitamin E (μg/mL)	16.53 ± 0.39**	14.42 ± 0.53
Serum vitamin E/TC ratio (μg/mmol)	2.82 ± 0.07	2.70 ± 0.08
Females		
Number of subjects	95	68
Vitamin E (μg/mL)	16.10 ± 0.49**	13.74 ± 0.54
Vitamin E/TC ratio (μg/mmol)	2.64 ± 0.09	2.50 ± 0.09
Males & Females		
Number of subjects	237	135
Vitamin E (μg/mL)	16.36 ± 0.31***	14.08 ± 0.38
Vitamin E/TC ratio (μg/mmol)	2.75 ± 0.05	2.60 ± 0.06

Values are expressed as Mean ± SEM or median and interquartile range. *=p<0.05, **=p<0.01, ***=p<0.001 comparison between patients and controls.
TC = toal cholesterol.

In general serum vitamin E and serum vitamin E/ total cholesterol ratio remained significantly higher in patients compared with controls for subgroups of dyslipidaemic patients, even for those sub-categories without the respective coronary risk factors.

Serum vitamin E levels did not differ between sub-categories of dyslipidaemic patients (p>0.05, Table 3) but vitamin E/ Total cholesterol ratio was significantly higher in patients with established CHD (p<0.01), hypertriglyceridaemia (p<0.05) and metabolic syndrome (p<0.05) compared with patients without established CHD, hypertriglyceridaemia and metabolic syndrome, respectively (Table 3).

Correlation between CHD risk factors and serum vitamin E status

In control subjects, serum vitamin E concentrations were positively associated with BMI (p<0.05), waist/ hip ratio (p<0.01), triglyceride (p<0.05) and total cholesterol (p<0.001). Vitamin E/ total cholesterol ratio was positively associated with waist/hip ratio (p<0.001, Table 4). In the patient group, vitamins E concentrations were positively associated with HDL (p<0.01), triglyceride (p<0.01) and total cholesterol (p<0.001). However, vitamin E/ total cholesterol ratio was negatively associated with HDL (p<0.05) and total cholesterol (p<0.001, Table 4).

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Table 3. Comparison of vitamin E between different subgroups of combined male and female dyslipidaemic patients segmented according to the possession of specific coronary risk factors.

	Number	Vitamin E (µ/ml)	Vitamin E/total Cholesterol ratio (µg/mmol)
Group			
Established CHD			
CHD+	55	16.19 ± 0.63[3]	3.02 ± 0.01[3]**
CHD-	182	16.41 ± 0.35	2.67 ± 0.06
Metabolic Syndrome		16.27 ± 0.39[3]	2.85 ± 0.07[3]*
MS+	142	16.49 ± 0.50	2.59 ± 0.08
MS-	95		
Diabetes Mellitus			
DM+	42	15.94 ± 0.75[3]	2.75 ± 0.13[3]
Impaired GT	21	15.09 ± 0.98	2.71 ± 0.18
DM-	174	16.61 ± 0.36	2.75 ± 0.06
Obesity			
Obese+	82	15.79 ± 0.52[3]	
Over weight	112	16.49 ± 0.43	
Normal weight	43	17.09 ± 0.81	
Hypertriglyceridaemia			
High T.G.	176	16.44 ± 0.36[3]	2.82 ± 0.06[3]*
Normal T.G.	61	16.11 ± 0.61	2.54 ± 0.10
Blood pressure			
High BP	76	16.41 ± 0.55[3]	2.79 ± 0.01[3]
Moderate BP	111	16.04 ± 0.43	2.75 ± 0.08
Normal BP	50	16.99 ± 0.70	2.68 ± 0.11
Calculated 10 year Coronary Risk			
High >30%	42	16.20 ± 0.40[3]	2.84 ± 0.12[3]*
Moderate 20-30%	54	16.34 ± 0.63	2.97 ± 0.11
Low <20%	141	16.41 ± 0.74	2.64 ± 0.07
Controls	135	14.08 ± 0.38	2.60 ± 0.06

Values are expressed as Mean ± SEM. 3=p<0.001 comparison between the groups including patients and controls, and *=p<0.05, **=p<0.01 comparison between sub-categories of the dyslipidaemic patients.

Table 4. Correlations (r) between serum vitamin E concentrations and vitamin E/total cholesterol ratio with individual coronary risk factors.

	Patients (n=238)		Controls (n=135)	
	Vitamin E (µg/mL)	Vitamin E/ TC (µg/mL)	Vitamin E (µg/mL)	Vitamin E/ TC (µg/mL)
Age	-0.08	0.07	0.17	-0.08
Fasting blood glucose	-0.04	0.03	0.10	-0.03
BMI	-0.09	-0.07	0.19*	0.08
Waist: Hip ratio	-0.05	0.07	0.28**	0.30***
HDL	0.05	-0.16*	0.14	-0.12
Triglyceride	0.21**	0.09	0.17*	0.03
Total cholestero	0.25***	-0.39***	0.50***	-0.13
Systolic BP	-0.03	0.05	-0.06	-0.10
Diastolic BP	0.10	0.06	0.05	0.00
CRP	0.02	-0.02	0.08	0.01

Correlations were assessed using Pearson correlation coefficients; *=p<0.05, **=p<0.01, ***=p<0.001. Non-normally distributed data such as serum triglyceride log transformed before using the Pearson correction. Between males and females as well as smokers and non-smokers, serum vitamin E and vitamin E/ total cholesterol ratio did not differ in control and patient subjects (p>0.05). TC = total cholesterol.

Vitamin E and metabolic syndrome

Within the dyslipidaemic group, serum concentrations of vitamin E and vitamin E/total cholesterol ratio did not differ significantly with accumulating of features of metabolic syndrome in male and female patients and the combined group (p>0.05, Table 5).

Table 5. Comparison of vitamin E status between dyslipidaemic patients with different features of the metabolic syndrom.

Number of Features	0	1	2	3	4	5
Males						
Number of Subjects	3	15	44	41	34	5
Vitamin E (μg/mL)	12.89±1.16	18.00±0.66	15.40±0.80	17.16±0.80	17.00±0.78	15.80±1.75
Vitamin E/TC ratio (μg/mmol)	2.49±0.11	2.77±0.19	2.55±0.12	3.00±0.13	3.00±0.15	2.83±0.46
Females						
Number of Subjects	7	13	17	21	22	15
Vitamin E (μg/mL)	17.86±2.36	15.20±1.24	17.63±1.35	16.66±0.91	15.70±0.93	14.14±1.15
Vit E/TC ratio (μg/mmol)	2.62±0.42	2.37±0.23	2.82±0.20	2.67±0.17	2.73±0.18	2.48±0.21
Males & Females						
Number of Subjects	10	28	61	62	56	20
Vit E (μg/mL)	16.37±1.88	16.70±0.87	16.02±0.62	16.99±0.61	16.49±0.60	14.55±0.96
Vit E/TC ratio (μg/mmol)	2.58± 0.29	2.59±0.15	2.62±0.10	2.89±0.10	2.89±0.12	2.61±0.19

Values are expressed as Mean ± SEM. One-way ANOVA used for comparison between the groups with different number of features of the metabolic syndrome. The diagnosis of the metabolic syndrome was made according to the ATP III Criteria for Identification of the Metabolic Syndrome (40).

Multifactorial analysis of Vitamin E

In order to evaluate the relationship between serum vitamin E concentrations and CHD risk factors (in the dyslipidaemic patients), and the effects of accumulating features of metabolic syndrome on serum vitamin E status, stepwise multiple regression analysis was undertaken.

Serum vitamin E

In dyslipidaemic patients, stepwise multiple regression analysis showed that only 1.9% of the variation in serum vitamin E concentrations was attributed to anti-hypertensive treatment (p=0.02, -1.4%), and anti-diabetic agents (p>0.05, -0.5%). In the subgroup with metabolic syndrome, 4.7% of the variation in serum vitamin E concentrations could be explained by serum triglyceride (p=0.009, +4.7%).

Serum vitamin E/ total cholesterol ratio

In dyslipidaemic patients, stepwise multiple regression analysis showed that 6.4% of the variation in serum vitamin E/ total cholesterol ratio was attributed to a history of established CHD (p=0.005, +2.9%) and hypertriglyceridaemia (p=0.01, +2.1%). Anti-hypertensive treatment (p=0.09, -0.8%) and metabolic syndrome (p>0.05, +0.6%) were not significantly associated. In the metabolic

syndrome subgroup, 5.7% of the variation in serum vitamin E/total cholesterol ratio could be explained by BMI (p=0.02, -3%), and diastolic blood pressure (p=0.03, -2.7%).

Discussion

Effect of age on serum vitamin E status

A significant increase in plasma α-tocopherol and cholesterol concentrations with age, but not the α-tocopherol: cholesterol ratio has been previously reported (31). In the present study, no significant correlation between age and either serum vitamin E or serum vitamin E: total cholesterol ratio was observed.

Effects of smoking on serum vitamin E status

In the present study, serum vitamin E status did not differ significantly between smokers and non-smokers. Relatively minor differences (or even no differences) between plasma α- and β-tocopherol levels in passive and active smokers as compared to their concentrations in non-smokers have been reported (32). This may result from the following reasons: 1) the secondary role played by these forms of vitamin E in the elimination of the free radicals derived from the tobacco smoke, 2) the increased regeneration rate of these forms and antioxidative mobilization in response to oxidative stress, 3) the association of smoking with change of plasma α- and γ-tocopherol levels was so slight that any differences in the experimental design such as describing smokers based on measuring urine cotinine or interviewing would have resulted in different outcomes (32).

Effects of dyslipidaemia on serum vitamin E status

Our results showed that serum vitamin E concentrations were higher in the patients than the controls. However as shown in lipid-standardised values, serum vitamin E/ total cholesterol ratio did not differ between patients and controls (Table 2). As vitamin E is a major lipid soluble antioxidant and is carried by cholesterol-rich lipoproteins, higher serum vitamin E concentration in the patients may be related to the higher serum cholesterol in patients than controls, and as we showed in lipid-standardised values, serum vitamin E / total cholesterol ratio did not differ between patients and controls.

Effects of CHD on serum vitamin E status

In the present study we found that serum vitamin E concentrations, after correction for serum total cholesterol, in the subgroup of patients with established CHD was significantly higher than that of patients without established CHD. Also, patients with calculated 10-year coronary risk greater than 30% had higher vitamin E/total cholesterol ratio compared with controls and patients with calculated 10-year coronary risk less than 20%.

Our finding is compatible with the results of other case-control studies on serum vitamin E levels (33-35), suggesting that there is no association between serum vitamin E levels and risk of coronary heart disease.

Although data from some prospective observational studies are compatible with possible cardiovascular benefits of vitamin E (15, 36-38), such studies are unable to control for the potential effects of un-known or unmeasured confounding variables that may explain all or part of observed associations. It may be, for example, that high vitamin E intake is only a marker for other dietary factors that are truly protective. Although it is plausible that the consumption of vitamin E-rich foods is protective, benefits may origin not from the foods' vitamin E composition but from other components found in these foods. Foods with a high antioxidant content also tend to contain minerals, flavonoids, and indoles, as well as several carotenoids. Antioxidant-rich diets tend to be low in saturated fat and cholesterol and high in fiber. It is also possible that intake of antioxidant vitamins from food or supplements is correlated with other unknown or unmeasured nondietary lifestyle behaviors that reduce the risk of cardiovascular diseases. Besides, increased intake of fruits, vegetables, and other antioxidant-rich foods should be promoted as part of a healthy diet because they provide nutritional benefits beyond any potential

antioxidant effect. Moreover, even if found to reduce risk of cardiovascular disease, vitamin E supplement use should be considered an adjunct, not an alternative, to established cardioprotective measures, such as smoking abstention, avoidance of obesity, adequate physical activity, and control of high blood pressure and hyperlipidemia.

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