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Association between vitamin A and E and apolipoprotein A and B levels in type 2 diabetes

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Objective. To determine the relationship between serum vitamin A and E and apolipoprotein levels in type 2 diabetic patients.

Setting. Shariati Hospital, Tehran, Iran.

Subjects and methods. One hundred and seventeen eligible type 2 diabetic patients who attended the Endocrine Research and Metabolism Center between 2002 and 2004 were enrolled in the study. Blood samples were collected after a 12 - 14-hour overnight fast for the measurement of serum levels of total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), apolipoprotein (apo) A1 and apoB, and vitamins A and E. Anthropometric indices were determined by physical examination. Data were analysed statistically using Pearson's coefficient, multiple regression, and partial and bivariate correlations.

Results. The mean body mass index (BMI) of the subjects was 27.4 ± 3.7 kg/m^2. The mean (± standard deviation (SD)) serum levels of vitamins A and E were 0.5 ± 0.1 µg/ml and 9.5 ± 2.6 µg/ml, respectively. There were no significant differences in the plasma levels of vitamins A and E in males and females. Mean serum levels of vitamins A and E were within the normal range for both sexes. Serum lipid levels (total cholesterol, triglyceride and apoB) correlated with serum levels of vitamin E ($p < 0.05$). Serum levels of vitamins A and E were also correlated ($p < 0.05$). Standardised vitamin E levels showed significant negative correlation with most studied lipid profiles ($p < 0.05$).

Conclusion. This study found that mean serum levels of the natural antioxidants vitamin E, and especially vitamin A, were close to the lower end of the normal range of these antioxidants in type 2 diabetics. Also, serum vitamin E and standardised vitamin E levels were important predictors of serum apoA1 levels in these patients.

Increased free radical activity has been reported in patients with type 2 diabetes mellitus (DM).[1] Tissue damage by free radicals (oxidative stress) is thought to be an important factor in the pathogenesis of DM and its complications.[2] Diabetic patients have been shown to have a high frequency of atherosclerosis, leading to increased risk of stroke and/or myocardial infarction.[3] Individuals with DM are 2 - 4 times more likely to die of heart disease than those without DM, and cardiovascular disease (CVD) is responsible for 80% of diabetes-related deaths.[4]

Lipoprotein abnormalities have been identified among the several risk factors that could account for the

increase in CVD in DM patients.[5] Recently, it has been found that reduced levels of apolipoprotein (apo) A1 are associated with an increase in coronary events.[6] In addition, apoA1 has been reported to be a better predictor of cardiovascular events than low-density lipoprotein (LDL)[7,8] and the strongest independent risk factor among high-density lipoprotein (HDL) parameters.[9,10]

Vitamin E functions *in vivo* as a chain-breaking antioxidant and prevents propagation of free radical damage in biological membranes.[11] Cell membrane damage is most effectively prevented by vitamin E, which reacts with peroxyl and hydroxyl radicals.[12] It has been found that administration of pharmacological doses of vitamin E is a useful tool to reduce oxidative stress and to improve insulin action in healthy and in type 2 DM patients.[13] Serum lipid levels have been correlated positively with vitamins A and E.[14] Thus, vitamins A and E, which have antioxidant properties, may moderate the effects of oxidative stress.

Several studies measuring serum concentrations of vitamins A and E in type 1 and 2 DM patients have shown different results, possibly owing to differences in patient selection or the effects of dietary management.[15-17] The objective of the present study was to investigate the relationship between serum levels of vitamin A and E and apoA1 and apoB in type 2 DM patients.

Materials and methods

The study was hospital-based. One-hundred and seventeen volunteer type 2 DM patients, 61 men and 56 women, aged 29 - 73 years, who attended the Endocrine Research and Metabolism Center at Shariati Hospital, Tehran, Iran, between the years 2002 and 2004 and who met the inclusion criteria, were enrolled in the study. The sample size was chosen based on the level of vitamin A, with 80% of statistical power to detect 20% differences at 5% significance level.

A patient had to meet the following criteria to be included in the study: willingness to co-operate with the study, normal hepatic and renal function, no history of myocardial infarction or other diabetic complications, not on vitamin supplements, thyroid hormones, oestrogens, progesterons, diuretics or β-blockers, and in the case of female patients, not pregnant. The subjects were fully informed of the purposes, procedures and hazards of the study. Written informed consent was obtained from all participants. The research protocol was approved by the Ethics Committee on Human Experimentation of Tehran University of Medical Sciences, Iran.

Data on medical history, type of medication, dietary supplementation and smoking habits were obtained from face-to-face interviews using a pre-tested standardised questionnaire. Height was measured with patients standing barefoot against a wall-fixed tape. Weight was measured with subjects wearing light clothing and standing barefoot on a platform scale, with a 1.0 kg subtraction to correct for the weight of clothing. Waist circumference was measured between the lower rib margin and the iliac crest. Hip circumference was measured at the largest circumference.[18] The body mass index (BMI) was calculated as weight/height^2 (kg/m^2) and the waist-to-hip ratio (WHR) as the ratio between the waist and hip circumferences. The WHR cutoffs for defining abdominal obesity are more than 0.95 and 0.80 for men and women, respectively.[19]

Subjects fasted overnight for 12 - 14 hours. Thereafter, at between 08h00 and 10h00, and before taking any oral hypoglycaemic agent(s), 20 ml blood samples were collected from each subject in tubes free from trace elements. Blood samples were centrifuged at 3 000 revolutions per minute for 10 minutes and aliquots of serum were transferred to polystyrene tubes which were immediately stored at –70°C until analysis. During the analysis, samples were protected from light and haemolysed samples were discarded. Serum alpha (α)-tocopherol (α-TOH) and vitamin A levels were determined using high-performance liquid chromatography according to the methodology described by Sanz and Santa-Cruz.[20] Lipid-standardised α-TOH (LS-α-TOH) was calculated as serum α-TOH concentration expressed per milligram triglycerides (TGs) plus cholesterol (µg/mg). Serum TGs and total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured enzymatically, and apoA1 and apoB were measured using the immunoturbidimetric method.[21] The intra- and inter-assay coefficient of variation was < 5%. The guidelines set by Fischenbach and Dunning[22] were used to determine the lipid profile. According to the standards established for vitamin A and E status,[23-25] serum retinol levels > 20 µg/ml and α-TOH > 5 µg/ml were considered normal. Intra- and inter-assay coefficient variation were 4.5% and 5% for retinol and 2% and 6% for α-TOH, respectively.

Descriptive and comparative statistics were performed for each variable. Analyses, such as the Pearson's correlation test, the Student's *t*-test, and multiple regression, were done. Log transformation was used to normalise the distribution of TG (log-TG). All other variables were normally distributed. A value of $p < 0.05$ was considered to be statistically significant. All data were analysed using SPSS version 11.5.

Results

Table I shows the mean (± standard deviation (SD)) age, duration of diabetes, BMI, WHR and smoking status of the study subjects. Fifty-two per cent of the

Table I. Age, duration of diabetes, BMI, waist/hip ratio and smoking status of the study subjects (mean ± standard deviation) (range)

Variables	Male (*N* = 61)	Female (*N* = 56)	*p*-value
Ages (yrs)	57.1 ± 10.4 (29 - 71)	49.1 ± 9.6 (32 - 73)	0.0001*
Duration of diabetes (yrs)	10.0 ± 8.0 (1 - 32)	7.8 ± 5.5 (1 - 20)	0.250
BMI (kg/m^2)	27.1 ± 3.5 (19.3 - 35.5)	27.7 ± 3.8 (19.0 - 37.5)	0.446
Waist/hip ratio	0.95 ± 0.1 (0.82 - 1.68)	0.84 ± 0.1 (0.65 - 0.98)	0.0001*
Smoking status (yes/no) (%)	12/49 (19.7)	4/52 (7.1)	0.049†

*Student's *t*-test.
†Chi-square test.

Table II. Serum levels of vitamin A and E, lipids and lipoproteins for the study subjects (mean ± standard deviation) (range)

Variables	Male (*N* = 61)	Female (*N* = 56)	Desirable range[29]
Vitamin E (µg/ml)	9.6 ± 2.8 (2.5 - 14.2)	9.5 ± 2.2 (5.3 - 14.8)	5 - 18
Vitamin A (µg/ml)	0.5 ± 0.1 (0.2 - 0.6)	0.4 ± 0.1 (0.3 - 0.6)	0.36 - 1.2
Standardised vitamin E* (µg/dl)	2.6 ± 0.8 (0.8 - 4.5)	2.6 ± 0.9 (1.1 - 5.0)	ND
Total cholesterol (mg/dl)	210 ± 41 (134 - 307)	227 ± 52 (110 - 369)	< 200
LDL-C (mg/dl)	139.6 ± 28.6 (92 - 217)	140.8 ± 35.3 (56 - 211)	< 130
HDL-C (mg/dl)	48.3 ± 8.6 (31 - 68)†	55.8 ± 9.2 (31 - 75)	Male: 35 - 65 Female: 35 - 80
Triglyceride (mg/dl)	171.0 ± 80 (60 - 570)	171.8 ± 23 (53 - 566)	< 150
apoA1 (mg/dl)	145.3 ± 19.1 (20 - 197)	167.6 ± 23.3 (70 - 218)	Male: 90 - 155 Female: 94 - 172
apoB (mg/dl)	127.6 ± 25.9 (80 - 202)	123.6 ± 31.9 (45 - 188)	Male: 55 - 100 Female: 45 - 110
TC/HDL-C ratio	4.4 ± 1.0 (2.8 - 8.2)	4.2 ± 1.1 (1.9 - 6.5)	3 - 6
LDL-C/HDL-C ratio	2.9 ± 0.6 (1.9 - 4.5)‡	2.6 ± 0.7 (0.9 - 4.2)	Low risk: M: 1.00 F: 1.47 Moderate risk: M: 3.55 F: 3.22

*Standardised vitamin E is calculated as µg serum α-tocopherol concentration expressed as per mg (cholesterol + triglyceride).
†Statistical significance $p < 0.0001$.
‡Statistical significance $p < 0.05$.
ND = not determined; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; TC = total cholesterol; apo = apolipoprotein.

subjects were male, and the mean age of females was significantly lower than that of males ($p < 0.0001$). The mean age of both sexes was 53.3 ± 10.7 years. The patients had been diabetic for between 1 and 32 years, with a mean duration of 8.9 ± 7.0 years. Based on BMI, 36% of male and 48% of female patients were overweight (BMI 26 - 30 kg/m²) and 21% of male and 25% of female patients were obese (BMI > 30 kg/m²). Based on WHR, 36% of male and 50% of female patients had abdominal obesity. Mean WHR was higher for men than for women ($p < 0.0001$). Eighty-five per cent of the patients were non-smokers and the proportion of smokers was higher among men than women ($p = 0.05$). There were no significant differences in the mean serum levels of vitamins A and E between smokers and non-smokers ($p \geq 0.5$).

Table II shows the serum levels of vitamins E and A, lipids and lipoproteins. With the exception of TGs, all the other variables had normal distributions. Six out of 61 patients (9.8%) had serum vitamin E levels < 5 µg/ml and 8 patients (13%) were below the desirable range for serum vitamin A levels.

The serum TG values for 25 males (41%) and 28 females (50%), and TC values for 27 males (44%) and 18 females (32%) were within the desirable range. The mean serum levels of cholesterol and LDL-C were higher than the desirable range. Thirty-one per cent of male and 45% of female patients had serum apoA1 levels higher than normal values. The mean serum levels of apoB in both sexes were higher than the desirable range, with 82% of male and 62.5% of female patients having serum apoB

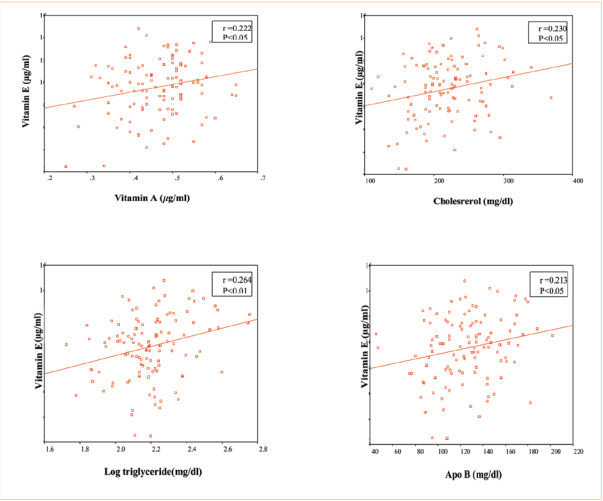

Fig. 1. Correlation between levels of vitamin E and vitamin A, cholesterol, log-triglyceride and apoB in the study group.

levels higher than the normal range. The mean ratio of TC/HDL-C was within the normal range. The mean ratio of LDL/HDL was higher than the desirable range. Therefore, 18% of male and 14% of female patients were at moderate risk of developing CVD. The serum HDL-C levels of 4 males (6.6%) and 1 female (1.8%) were below the desirable range. Women had significantly higher serum HDL-C levels ($p < 0.0001$) and lower LDL-C/HDL-C ratios ($p < 0.05$) than men.

Vitamin E levels were positively correlated with lipid profiles (Fig. 1). Significant correlations were observed between serum vitamin E levels and serum levels of vitamin A, cholesterol, log-TG, and apoB ($r = 0.22$, $p < 0.05$; $r = 0.23$, $p < 0.05$; $r = 0.26$, $p = 0.004$; $r = 0.21$, $p < 0.05$, respectively).

Standardised serum vitamin E levels showed significant negative correlations with the serum levels of log-TG, cholesterol, apoB, LDL and the ratio of LDL/HDL and TCH/HDL ($r = -0.53$, $p < 0.0001$; $r = -0.45$, $p < 0.0001$; $r = -0.40$, $p < 0.0001$; $r = -0.30$, $p < 0.0001$; $r = -0.38$, $p < 0.0001$; $r = -0.50$, $p < 0.0001$, respectively (Fig. 2).

Multiple regression analysis showed significant correlations between serum apoA1 levels and the levels of log-TG ($p = 0.05$), cholesterol ($p < 0.05$), vitamin E ($p < 0.05$), standardised vitamin E ($p < 0.05$) and HDL ($p < 0.0001$). Similar analysis for the serum apoB levels and the levels of other variables showed only significant correlation with the LDL levels ($p < 0.0001$).

Discussion

The limitations of this study were not collecting dietary intake data or having a control group for comparison. Probable sources of error were duration of the study for collecting data. Based on BMI, 27 patients (23%) were obese. Obese adults are considered to be at risk for developing chronic diseases.[26] A 20% increase in

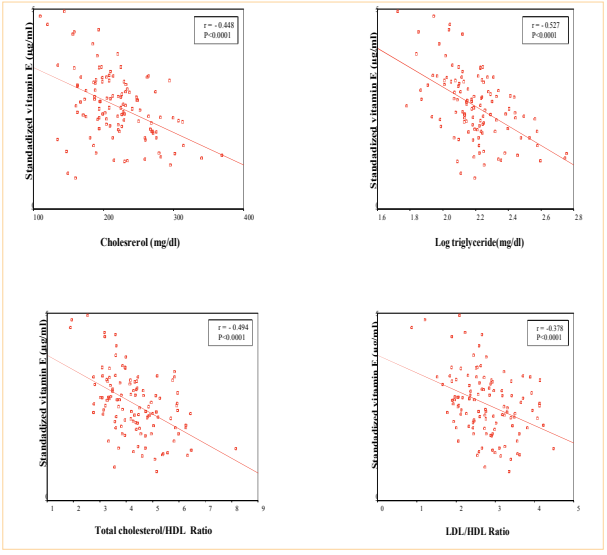

Fig. 2. Correlation between levels of standardised vitamin E and cholesterol, log-triglyceride, TCH/HDL and LDL/HDL ratio in the study group.

body weight substantially increases the risk of lipid disorders and type 2 DM.[26] A strong association exists between obesity and DM.[27] In fact, obesity can be considered the most 'environmental' determinant in the development of diabetes.[28] Obesity is associated with hyperinsulinaemia and insulin resistance in diabetes.[29] Waist circumference and WHR are similarly correlated with the risk of coronary heart disease (CHD).[30] In our study group, 43% of patients had abdominal obesity and were at risk of developing CHD.

The relationship between obesity and insulin resistance is well established, but the molecular mechanisms involved are poorly understood.[31] Moreover, obesity is associated with accelerated oxidative stress[32-33] which aggravates vascular changes and induces early CVD.[34] It has been demonstrated that oxidative stress is higher in obese diabetic than in obese non-diabetic subjects.[35-36]

Impaired glucose tolerance is often associated with obesity and raised levels of plasma free fatty acids.[37] Previous investigators have demonstrated that patients with type 2 DM have higher serum vitamin A concentrations than normal subjects.[15-17] Tavridou *et al.*[2] also found serum vitamin A levels to be higher in subjects with impaired glucose tolerance (IGT). Their data suggest that higher serum vitamin A concentrations exist in subjects before the onset of diabetes. Sasaki *et al.*[17] have shown that the increased vitamin A levels in type 2 DM occur independently of lipid status. In their study, vitamin A levels showed a positive correlation with apoC-II in both diabetic and control subjects. Furthermore, Tavridou *et al.*[2] found positive correlations between the serum vitamin A levels and both serum triglyceride and cholesterol levels in IGT patients. The average serum vitamin A and E levels were within the desirable range in our study (Table II).

Gouado *et al*.[14] also found a strong correlation between vitamins A and E in healthy people. In the present study the serum vitamin A levels did not show any significant correlation with the investigated serum variables, except for vitamin E, and this relationship remained significant after adjusting for serum log-TG concentrations. The significant links observed between vitamin A and E may suggest similar pathways of absorption and blood transportation of these vitamins, with lipoproteins or other specific binding proteins.[38]

In our study, the serum vitamin E levels showed significant correlation with serum cholesterol, log-TG and apoB levels (Fig. 1), which is similar to the results of Gouado *et al*.,[14] whereas the standardised vitamin E levels showed significant negative correlations with most of the serum levels of lipid profiles (Fig. 2). Furthermore, multiple regression analysis showed significant correlations between serum levels of apoA1 and vitamin E, standardised vitamin E and HDL. These findings may suggest that serum levels of vitamin E, standardised vitamin E and HDL could be considered predictors of apoA1 status.

Farvid *et al*.[39] found that co-supplementation with vitamin E, vitamin C, magnesium and zinc increased serum levels of HDL-C and apoA1 after 3 months of intervention in type 2 DM patients. In their study, positive correlation existed between changes in serum vitamin E, HDL-C and apoA1. HDL may protect against CHD through reverse cholesterol transport and also antioxidant and anti-inflammatory properties.[6] Because of its antioxidative role, vitamin E may protect HDL-C and apoA1 from oxidation, and increase the rate of reverse cholesterol transport.

Serum levels of apoB and apoA1 and the ratio of apoB/apoA1 have been reported to be better predictors of cardiovascular events than LDL-C alone.[8] They have even been shown to retain their predictive power in patients receiving lipid-modifying therapy.[8] In addition, apoA1 levels have been reported as the strongest independent risk factor indicator among HDL parameters.[9,10] Low levels of HDL-C increase cardiovascular risk and are associated with insulin resistance, hypertension, DM, high levels of TG and LDL-C, and a precoagulant state.[39] As potent antioxidants, serum carotenoids may also play a protective role in the development of chronic diseases including CVD.[40-42] Beta-carotene could decrease the oxidative susceptibility of LDL.[43] Serum carotenoid levels are inversely associated with type 2 DM and impaired glucose metabolism.[44] As a major water-soluble antioxidant, vitamin C acts as the first line of defence during oxidative stress. Its blood concentration is solely dependent on dietary intake, but once it is oxidised it can be reduced back to its antioxidant form by glutathione.[45] Vitamin C can regenerate vitamin E through redox cycling, thus it plays an important role in protein thiol group protection against oxidation.[46]

Supplementation with vitamin E or a combination of vitamin E and other nutrients or non-nutrients could increase serum HDL-C and apoA1 levels.[31,47,48] Meksawan *et al*.[49] found that subjects on a low-fat diet (19%) had significantly lower serum HDL-C and apoA1 levels than those on a high-fat diet (50%). Therefore, it seems that manipulating levels of vitamin E intake, with or without other nutrient manipulation, could impact favourably on apolipoprotein profile, especially apoA1, in terms of decreasing CVD risk.

Vitamin E is the major lipid-soluble antioxidant in cell membranes and plasma lipoproteins.[37] It reacts with the lipid peroxide radicals formed by peroxidation of polyunsaturated fatty acids before they can establish a chain reaction. The tocopheroxyl free radical product is relatively non-reactive and can be reduced back to tocopherol by reaction with vitamin C from plasma. Tocopheroxyl could penetrate further into cells and potentially propagate a chain reaction.[37] Although studies have shown an association between high blood concentrations of vitamin E and a lower incidence of atherosclerosis, this may explain why the effect of high doses of vitamin E has been disappointing. Standardised vitamin E may show a better relationship with lipid profile than vitamin E.

In conclusion, mean serum levels of vitamin E, and especially vitamin A, were close to the lower end of the desirable range in our study group, but mean serum apoB levels were higher than the desirable range. Serum levels of vitamin E and standardised vitamin E may be considered predictors of serum apoA1 levels. These findings need confirmation by means of further research. More studies are required to demonstrate the benefits of vitamin A and E supplementation in improving the anti-oxidant status of diabetics by increasing serum levels of apoA1 and HDL in order to decrease the risk of CVD in these patients.

This study was supported by grant number 132-9261 from the Research Undersecretary of Tehran University of Medical Sciences. We are indebted to the patients for their co-operation.

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