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Antioxidant Vitamins and Lipid Peroxidation in Patients with Type 1 Diabetes Mellitus

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Abstract

It has been postulated that oxidative stress may be increased and antioxidant defenses reduced in diabetes patients. Twenty-four patients with type 1 diabetes mellitus (DM) (12.8±1.7 years) and 24 nondiabetics (12.5±2.1 years) were included in this study. Serum total cholesterol and LDL-cholesterol levels were significantly higher in diabetic than in nondiabetic control subjects, but serum levels of triglyceride, retinol, tocopherol, and β-carotene were significantly lower. Both β-carotene and tocopherol levels inversely correlated with HbA_{1c}, suggesting perhaps that low serum antioxidant level enhance the glycosylation of hemoglobin. Subjects with type 1 DM had lipid peroxide levels similar to those of nondiabetic control subjects, suggesting that peroxidation of circulating lipid is not increased in uncomplicated diabetics. The correlation between antioxidants and serum lipids were as follows: retinol and LDL (r=-0.36, p=0.019); retinol and total cholesterol (r=-0.35, p=0.020), tocopherol and LDL (r=-0.47, p=0.002); tocopherol and cholesterol (r=-0.49, p=0.001); β-carotene and LDL (r=-0.51, p=0.001). Overall, the results of this study were that serum lipid peroxide in patients with type 1 DM was similar to those of control subjects and antioxidants such as retinol, tocopherol and β-carotene were lower than those of nondiabetic control subjects, and negatively correlated with serum total cholesterol and LDL-cholesterol.

Key words: type 1 diabetes mellitus, antioxidant vitamins, lipid peroxidation

INTRODUCTION

Diabetes is associated with a markedly increased risk of atherosclerotic cardiovascular disease (1). However, the mechanisms by which diabetes enhances atherosclerosis and its complications has not been well established. Subjects with type 1 diabetes mellitus (DM) also have an increased risk of cardiovascular disease (2), despite the absence of the central obesity/insulin resistance syndrome and the presence of normal or even high levels of HDL-cholesterol (3,4).

Recently, attention has focused on oxidized lipids, metabolites that have been implicated in a wide range of diseases. Originally, Sato et al. (5) reported that lipid peroxides were elevated in the plasma of diabetic patients, but the increase noted was entirely due to patients with angiopathy. Support for this observation was provided by Jennings et al. (6), who noted that only in diabetic patients with microvascular disease was the concentration of circulating conjugated dienes increased. And Yagi (7) determined the level of peroxides in plasma obtained from a variety of human subjects and reported that peroxide concentrations were elevated in diabetic patients.

Strategies for reducing the oxidative stress may include the supplementation of antioxidant micronutrients (vitamins E and C, β-carotene). The different susceptibility of diabetic patients to microvascular and macrovascular complications may be a function of endogenous antioxidant status (8). Increased susceptibility to oxidation has been demonstrated recently in diabetic patients with poorly controlled type 1 diabetes mellitus (9). The reduction of glutathione peroxidase, glutathione reductase activities and vitamin C levels confirms the existence of oxidative stress in type I diabetes (10). Vitamin E supplementation significantly lowers blood lipid peroxide and lipid levels in diabetic patients (11).

This study was conducted to determine whether lipid peroxide levels in serum and urine, lipid profiles, and antioxidant vitamins such as retinol, tocopherol and β-carotene in patients with type 1 diabetes mellitus would differ from controls, and to find out the correlations among those factors.

MATERIALS AND METHODS

Subjects

Twenty-four patients with type 1 diabetes mellitus (11 males, 13 females) who have been attending several hospitals in Taegu and 24 apparently healthy nondiabetic subjects (12 males, 12 females) were studied (Table 1). They were matched in age (12.7±1.71 vs 12.5±2.14 years) and weight (BMI 18.9±4.7 vs 20.3±5.4 kg/m^2).

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Table 1. Clinical characteristics of study subjects

Characteristics	Control	Type 1 DM[1)]
n	24	24
Gender (M/F)	12 / 12	11 / 13
Age (years)	12.5± 2.1	12.8 ± 1.71
Height (cm)	139.8±11.8	146.5 ± 8.0*
Weight (kg)	41.1±15.1	40.9 ±11.2
BMI[2)] (kg/m^2)	20.3± 5.4	18.9 ± 4.8
Duration (years)		8.2 ± 0.21
FBS[3)] (mmol/L)	5.1± 0.8	10.1 ± 0.13**
HbA_1c[4)] (%)	5.8± 2.0	10.07± 3.3**
Hemoglobin (g/L)	133.2±12.1	136.3 ± 9.2
TP[5)] (g/L)	75.8±16.3	71.6 ±14.2
Albumin (g/L)	37.3±17.2	45.0 ± 9.1
Retinopathy	0 of 24	1 of 24
Neuropathy	0 of 24	4 of 24

Values are Mean±S.D.
*p<0.05, **p<0.01
[1)]DM: Diabetes Mellitus, [2)]BMI: Body Mass Index (kg/m^2),
[3)]FBS: Fasting Blood Sugar, [4)]HbA_{1c}: Glycosylated Hemoglobin,
[5)]TP: Total Protein

Sample collection and handling

Overnight, fasting blood samples were collected by venipuncture and the separated serum was protected from light and frozen for later analysis. Hemoglobin A_{1c} (HbA_{1c}) was determined by microcolumn chromatography, and blood glucose by the glucose oxidase method. Serum was stored at -70°C and used within 1 month for analysis of lipid profile, lipid peroxidation, and antioxidant vitamin levels. The injection of insulin was withheld until the blood was drawn on the morning of the study to avoid possible hypoglycemic reactions due to fasting. Urine samples were collected for 24 hours.

Nutritional status

Daily nutrients intakes were determined by indirect method (12).

Measurement of serum lipids

Serum cholesterol levels were determined using a commercial kit (Asan Chemical Co.) based on modification of the cholesterol oxidase method (13). HDL-cholesterol concentration was determined using the same enzymatic method and LDL-cholesterol was calculated according to the Friedwald calculation (14). Serum triglyceride levels were measured enzymatically using a kit from Asan Chemical Co., a modification of the lipase-glycerol phosphate oxidase method (15).

Thiobarbituric acid test of urine and serum

The levels of thiobarbituric acid reactive substances (TBARS) in urine and serum samples were measured by the modified filtration procedure of Tarladgis et al. (16). Duplicate samples of 0.5 ml urine were mixed thoroughly with 3 ml of 5% trichloroacetic acid (TCA) and 1 ml of 0.06 M TBA solution in screw-capped culture tubes. The mixtures were heated in an 80°C waterbath for 90 min, cooled to room temperature, and centrifuged at 1,360×g for 15 min to remove fine precipitates. The absorbance of the supernatant was read at 535 nm using a Beckman (Fullerton, CA) Model DU-8 spectrophotometer. Malondialdehyde (MDA) standards were freshly prepared from tetramethoxypropane (TMP) and treated in the same way as the test samples. The amounts of TBARS in urine and serum were expressed as equivalents of MDA.

Measurement of retinol and tocopherol

Serum retinol and tocopherol was measured as described by Bieri et al. (17). Total lipid extract from 0.1 ml serum containing internal standards (tocopheryl acetate and retinyl acetate, respectively) was injected into HPLC (Shimadzu SCL 10A) with reverse phase C_{18} column developed with 100% methanol. A flow rate of 1.0 ml/min was used with the UV detector set (Shimadzu) at 292 nm.

Measurement of β-carotene

Serum β-carotene was measured as described by Kim (18). Serum β-carotene was determined by HPLC. Serum (0.7 ml) was placed in 1 ml methanolic KOH (1 mole/L solution of KOH in absolute methanol) and shook in a waterbath (60°C) for 30 min. After cooling, the mixture was extracted with 2 ml of petroleum ether (PE) and centrifuged at 1,000 ×g for 10 min. This extraction procedure was repeated and 100 µl of combined PE extract was filtered through a 0.2 µm membrane filter and used for analysis. The HPLC system was Shimadzu SCL 10 A with UV detector set at 450 nm. A C_{18} revere phase column was used. The solvent was acetonitrile : methylene chloride : methanol (70 : 20 : 10, v/v), run at 1.7 ml/min.

Statistical analysis

Statistical significances were determined using the SPSS package. Values are given as the means±S.D. Comparisons between diabetes and control group were made using the non-parametric Mann-Whitney test. Correlation analysis was used for detecting relationships among serum lipid values, TBARS, and various serum antioxidant levels.

RESULTS AND DISCUSSION

Clinical characteristics of subjects are shown in Table 1. Fasting blood sugar and glycosylated hemoglobin (HbA1c) were significantly higher in diabetes than in controls (p=0.01). Duration of diabetes was 8.2 years in diabetes group. Four of the diabetic patients had neuropathy and one had retinopathy.

Nutrient intakes of subjects are shown in Table 2. There was no significant difference between diabetics and controls.

Serum lipid profiles of control and diabetics are shown in Table 3. Total cholesterol (TC) and LDL-cholesterol (LDL) were significantly higher in diabetics than in controls (p=0.01). But triglyceride was significantly lower in diabetics than in controls (p=0.01). Among many factors, elevated lipids and lipid peroxide levels in blood are major risk factors in the development of cardiovascular disease in diabetic patients. Our finding of increased TC and LDL in diabetics confirms that the risk of cardiovascular disease is higher.

Thiobarbituric acid reactive substances (TBARS) concentrations for controls and type 1 DM subjects are shown in Table

Table 2. Nutrients intakes in control and subjects with type 1 diabetic mellitus

Nutrients	Male		Female	
	Control	Type 1 DM[1)]	Control	Type 1 DM
Energy (Kcal)	2366.6 ±407.8	2224.9 ±302.4	2014.3 ±462.8	2051.7 ±2719
Protein (g)	90.8 ± 18.6	91.1 ± 13.7	80.6 ± 18.6	87.1 ± 14.1
Fat (g)	54.1 ± 14.2	52.8 ± 9.1	65.6 ± 14.5	52.2 ± 13.8
Carbohydrate (g)	379.2 ± 62.1	346.2 ± 57.1	320.3 ± 74.6	308.5 ± 42.2
Fe (mg)	17.5 ± 3.5	19.3 ± 3.0	16.6 ± 3.7	18.9 ± 3.0
Calcium (mg)	792.3 ±136.4	850.6 ±175.5	721.4 ±211.3	813.6 ± 170.1
Vit. A (R.E.)	653.9 ±138.3	736.8 ±163.4	599.6 ±194.8	694.5 ± 128.6
Vit. B_1 (mg)	1.34± 0.19	1.32± 0.18	1.18± 0.25	1.20± 0.13
Vit. B_2 (mg)	1.31± 0.28	1.43± 0.28	1.19± 0.35	1.36± 0.27
Niacin (mg)	23.06± 3.81	17.66± 7.26	20.3 ± 4.17	16.15± 5.96
Ascorbic acid (mg)	51.69± 15.6	62.55± 18.3	49.2 ± 21.5	62.43± 15.41
PFC ratio[2)]	15 : 20 : 65	16 : 22 : 62	16 : 20 : 64	17 : 23 : 60

Values are Mean±S.D.
[1)]DM: Diabetes Mellitus, [2)]PFC ratio: protein : fat : carbohydrate ratio

Table 3. Serum lipid profiles of control and Type 1 DM subjects

	Control	Type 1 DM[1)]
Total Cholesterol (mmol/L)	3.63±0.49	4.60±0.79**
HDL-Cholesterol (mmol/L)	1.06±0.34	1.16±0.26
LDL-Cholesterol (mmol/L)	1.88±0.48	3.07±0.82**
Triglyceride (mmol/L)	1.54±0.81	0.98±0.51**
Atherogenic Index[2)]	3.82±0.29	4.25±0.32

Values are Mean±S.D.
**p<0.01
[1)]DM: Diabetes Mellitus
[2)]Atherogenic Index: (total-cholesterol - HDL-C)/HDL-C

Table 4. Serum and urine malondialdehyde concentration of control and type 1 DM subjects

	Control	Type 1 DM[1)]
Serum TBA (nmol/ml)	2.82±1.39	3.03±1.14
Urine TBA (nmol/ml)	15.32±5.84	18.67±9.70

Values are Mean±S.D.
[1)]DM: Diabetes Mellitus

4. There was no significant difference between controls and diabetics, but the diabetics had slightly higher TBARS concentrations than controls. Lipoprotein lipid peroxides are expressed in terms of malondialdehyde (MDA) equivalents (19). MDA, an easily measurable aldehyde, is one of the many compounds induced by cell membrane polyunsaturated fatty acids (PUFA) attacked by free radicals. MDA can either be catabolized totally in the organism (20), or alter the lysine residues of LDL apo B100 (21). The modified LDL would then be recognized by a specific macrophage receptor (22), resulting in the formation of cholesterol-rich foam cells which are constituents of the artheromatous plaque (23-25). For this reason, we measured serum and urine MDA as thiobarbituric acid reactive substances (TBARS). Howerever, our study showed no significant difference in lipid peroxide levels between diabetics and non-diabetic control subjects, suggesting that peroxidation of circulating lipid is not increased in uncomplicated diabetics.

Serum antioxidant vitamins in controls and type 1 DM subjects are shown in Table 5. Serum retinol concentrations were significantly decreased in diabetic patients ($p<0.05$) compared with the control group. Serum α-tocopherol concentration and β-carotene concentration in diabetic patients were also significantly lower than the control group at $p<0.01$, $p<0.001$, respectively.

Table 5. Serum antioxidant vitamins in control and type 1 DM subjects

	Control	Type 1 DM[1)]
Retinol (μmol/L)	1.31±0.39	1.06±0.24*
α-Tocopherol (μmol/L)	25.59±7.15	20.32±5.01**
β-Carotene (μmol/L)	0.42±0.12	0.21±0.06***
Tocopherol/cholesterol	7.05±5.93	4.23±1.47*

Values are Mean±S.D.
*p<0.05, **p<0.01, ***p<0.001
[1)]DM: Diabetes Mellitus

Tissue damage in arteries and organs such as the eyes and kidneys is characteristic of diabetes mellitus both in humans and experimental models (26-28), making diabetics more prone to atherosclerosis, blindness, and renal failure. It has been reported that antioxidants in certain tissues of diabetic rats are decreased (29-31), which may reflect the increased occurrence of oxidation and a decrease in capability to protect against oxidation. That is, increased lipid peroxide might be related to tissue damage.

Our finding of decreased antioxidant vitamin concentrations in subjects with type 1 DM suggests that antioxidant defenses are reduced in diabetics, and total peroxyl radical trapping potential in subjects with type 1 DM are reduced.

Reduced antioxidant defenses in poorly controlled subjects with IDDM may thus, in part, account for the increased susceptibility of LDL from subjects with IDDM to oxidative modification (9). Because peroxyl radical trapping capacity also is likely to be reduced in the millieu of the artery wall, where lipoproteins appear to be oxidized during atherogenesis, and thus antioxidant therapy may be of value in reducing the markedly accelerated atherosclerosis seen in diabetes. This hypothesis shoud be tested by a clinical trial.

The correlations between serum lipids, TBARS and antioxidant vitamins are shown in Table 6. HbA_1c and TC, LDL-C, HDL-C were positively correlated. HbA_1c and urine TBARS

Table 6. Correlations between serum lipids, TBARS and antioxidant nutrients in control and subjects with type 1 diabetes mellitus

	HbA_{1c}[1)]	TC[2)]	TG[3)]	HDL-C	LDL-C	AI[4)]	S-TBARS[5)]	U-TBARS[6)]	Retinol	β-Carotene
TC	0.651***									
TG	-0.321*	-0.268								
HDL-Chol.	0.390*	0.385**	-0.487**							
LDL-Chol.	0.686**	0.943**	-0.380*	0.288						
AI	-0.038	0.180	0.487**	-0.769**	0.209					
S-TBARS	-0.064	-0.242	-0.203	0.093	-0.154	-0.218				
U-TBARS	0.404**	0.362*	-0.061	0.192	0.332*	-0.058	-0.074			
Retinol	-0.252	-0.354*	0.209	-0.257	-0.365*	0.117	-0.095	0.054		
β-Carotene	-0.637**	-0.484**	0.129	-0.149	-0.507**	-0.119	0.039	-0.099	0.366*	
Tocopherol	-0.027	-0.149	-0.064	-0.105	0.036	-0.048	-0.324	0.183	0.671**	0.415**

*p<0.05, **p<0.01, ***p<0.001
[1)]HbA_{1c}: glycosylated hemoglobin, [2)]TC: total cholesterol, [3)]TG: triglyceride, [4)]AI: atherogenic index = (total-cholesterol - HDL-C)/HDL-C
[5)]S-TBARS: serum thiobarbituric acid reactive substances, [6)]U-TBARS: urine thiobarbituric acid reactive substances

were positively correlated ($p<0.01$). LDL-C and retinol, β-carotene were negatively correlated at $p<0.05$, $p<0.01$, respectively. Retinol, β-carotene and α-tocopherol were positively correlated ($p<0.05$, $p<0.01$, respectively).

Overall, the results of this study were that antioxidants such as retinol, tocopherol and β-carotene in patients with type 1 DM were lower than nondiabetic control subjects, and negatively correlated with serum cholesterol and LDL-C. Both β-carotene and tocopherol levels were inversely correlated with HbA_{1c}, suggesting perhaps that low serum antioxidant level enhance the glycosylation of hemoglobin. Subjects with type 1 DM had lipid peroxide levels similar to those of nondiabetic control subjects, suggesting that peroxidation of circulating lipid is not increased in uncomplicated diabetics.

However, this study must be considered as preliminary. The relatively small number of subjects studied does not permit for full analysis. A number of questions raised deserve further study. In particular, positive correlation between HbA_{1c} and urine TBARS is intriguing and potentially important, given the increasing recognition of oxidized lipids as atherosclerotic risk factors. Further studies are planned to investigate the oxidation of specific lipoprotein fractions and to determine if plasma lipid peroxidation correlates both cross-sectionally and prospectively with cardiovascular disease in diabetics.

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