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Relationship between lipid profile and lipid hydroperoxide levels in early stage type l diabetic patients

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ABSTRACT

We studied 51 early stage type 1 diabetic patients and 48 healthy normolipidemic age-matched controls to examine the influence of lipid profile on lipid hydroperoxide levels. The type 1 diabetic patient group consisted of 12 prepubertal children, 18 adolescents, and 21 young adults who were free from any clinical evidence of retinopathy, nephropathy, or neuropathy. We determined serum triglyceride, total cholesterol, HDL (high-density lipoprotein)-cholesterol, LDL (low-density lipoprotein)-cholesterol, VLDL (very low-density lipoprotein)-cholesterol, apolipoprotein A-1, apolipoprotein B, lipoprotein (a) levels as markers of lipid profile, and lipid hydroperoxide levels (LHP) as markers of lipid peroxidation, and tried to show the relation, if any, between lipid profile and lipid peroxidation. Serum lipid profiles of three groups of diabetic patients were not different compared to their controls. However, LHP levels were increased significantly in adolescent and young adult type 1 diabetic patients compared to their controls. The significant increase in LHP levels in these two groups of patients was independent from their lipid profile parameters except triglycerides. The lack of difference in LHP levels in prepubertal diabetic patients and their controls might be explained by intense hormonal and metabolic changes during puberty.

Glycation and free radical reactions may play an important role in diabetic complications (2, 8). Long-term vascular complications still represent the main cause of morbidity and mortality in diabetic patients (8). Long diabetes duration and poor glycemic control have been shown to be the most important risk factors for the development of microvascular disease in these patients (18). However, children and adolescents with type 1 diabetes normally do not show evident clinical manifestations of microangiopathy until later in adolescence or early in adult life (14).

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Plasma thiobarbituric acid reactive substances (TBAR's) (9, 10, 11, 13, 15), plasma malondialdehyde (MDA) (1, 5, 12), and plasma lipid hydroperoxide (LHP) (3, 4, 10, 15, 17) levels were determined as lipid peroxidation markers, and have been reported to be increased in type 1 diabetic patients compared to their controls. The increase in lipid peroxidation markers has been implicated in the pathogenesis of diabetic complications (12). Significantly higher values of TBAR's in serum, which provide an indirect measurement of lipid peroxidation and decreased erythrocyte antioxidant enzyme activities, have been observed in adult diabetic patients (9, 13), although no differences have been found in these activities in pediatric diabetic patients compared with control subjects (11). Furthermore, in children and adolescents, MDA levels continued to rise over the course of the disease, indicating

overproduction of free radicals and leading to lipid peroxidation and cell oxidative injury, which is considered by some authors to be related to the development of diabetic complications (20, 21).

We could find no study that measured total cholesterol, HDL-cholesterol, LDL- cholesterol, VLDL-cholesterol, apolipoprotein A-1, apolipoprotein B, lipoprotein (a), LHP together in early stage type 1 diabetic patients. So we aimed to investigate these parameters together in early stage type 1 diabetic patients.

MATERIALS AND METHODS

Human subjects. The sample groups consisted of 51 type 1 diabetic patients, and 48 healthy, age-matched normolipidemic voluntary subjects (Table 1). Diabetic patients were classified under three groups as prepubertal, adolescent, and young adult. The attainment of final adult height, as taken from the case-notes growth chart, was used as the criterion of adulthood, and those referred to as prepubertal were classified according to the Tanner classification (stage 1–2) (16). Those in-between were considered to be adolescents. General physical examination was carried out in all participants. Blood pressure, heart rate, height, and weight were recorded and the pubertal status was determined after assessment by a consultant endocrinologist. Clinical characteristics of early stage type 1 diabetic patients and their controls were given in Table 1. Patients with microalbuminuria, coronary heart disease, or hypertension were excluded from the study. Type 1 diabetic patients were free from any clinical evidence of retinopathy, nephropathy, and neuropathy.

None of the patients was known to suffer from an acute illness or chronic inflammatory condition at the time of study. Type 1 diabetic patients were not taking any medication that might have adverse effects on the tests performed. All subjects were non-smokers. All samples were taken in fasting state in the morning. A period of 10 min rest in the recumbent position was allowed before blood sampling. The samples were obtained from antecubital vein using 21 gauge sterile needle and blood was allowed to flow freely into Vacutainer tubes containing heparin, EDTA, or no additive. For apolipoprotein A-1, and apolipoprotein B, serum samples were stored at $-70°C$ until analysis, all other parameters including lipoprotein (a) were determined on the same day of collection. Participants gave informed consent, and procedures followed were in accordance with the Ethical Committee of Istanbul Faculty of Medicine.

Chemicals. All reagents were analytical grade. Hydrogen peroxide, xylenol orange and sorbitol were purchased from Sigma (St. Louis, MO, USA); H_2SO_4 was purchased from Riedel-de Haen (Seelze, Germany); ammonium ferrous sulfate was purchased from Merck (Darmstadt, Germany). Deionized water was used in all analytical procedures. All reagents were stored at +4°C. The reagents were equilibrated at room temperature for 0.5 h before use.

Table 1 *Clinical characteristics of early stage type 1 diabetic patients and their controls*

	Prepubertal diabetic group (n=12)	Control group 1 (n=14)	Adolescent diabetic group (n=18)	Control group 2 (n=15)	Young adult diabetic group (n=21)	Control group 3 (n=19)
Age (years)	11.14 ± 1.21	10.17 ± 2.32	15.33 ± 1.49	15.8 ± 1.78	19.36 ± 1.53	19.34 ± 1.01
Sex (M/F)	6/6	8/6	10/8	8/7	9/12	9/10
Height (m)	1.50 ± 0.14	1.40 ± 0.17	1.64 ± 0.12	1.62 ± 0.18	1.63 ± 0.17	1.67 ± 0.18
Weight (kg)	45.14 ± 6.63	42.60 ± 5.32	57.94 ± 8.85	56.66 ± 8.25	63.19 ± 10.1	60.47 ± 9.71
Diabetes duration (years)	3.57 ± 1.62	-	3.28 ± 1.36	-	4.88 ± 0.99	-
Glucose (mmol/l)	6.44 ± 0.96	5.04 ± 0.52	6.85 ± 0.93	5.01 ± 0.69	6.91 ± 0.62	5.00 ± 0.54
HbA_{1c} (normal < 7.2)	7.56 ± 0.51	-	7.53 ± 0.49	-	7.51 ± 0.60	-
Fructosamine (mmol/l)	3.09 ± 0.68	-	3.06 ± 0.67	-	3.08 ± 0.63	-
Systolic blood pressure (mm Hg)	109 ± 9	110 ± 9	113 ± 10	112 ± 9	112 ± 11	118 ± 7
Diastolic blood pressure (mm Hg)	67 ± 8	68 ± 7	70 ± 9	74 ± 9	71 ± 8	73 ± 8

Values are represented as arithmetic means ± S. D.

Apparatus and laboratory methods. All centrifugation procedures were performed with Jouan G 412; plasma LHP levels were measured by a spectrophotometer (Ultrospec 4050 LKB). Apolipoprotein A-1, apolipoprotein B, and lipoprotein (a) levels were determined by turbidimetry using monoclonal-immunoprecipitation methods (Behring turbitimer). Plasma glucose, total cholesterol, and triglyceride levels were determined colorimetrically on the DAX-72 discrete autoanalyzer (Technicon, Bayer Diagnostics, New York). Plasma direct high density lipoprotein (HDL)-cholesterol (Randox Lab. Ltd., Antrim, UK), and fructosamine levels were determined colorimetrically on the RA-XT discrete autoanalyzer (Technicon, Bayer Diagnostics, Dublin). Low density lipoprotein (LDL)-cholesterol and very low density lipoprotein (VLDL)-cholesterol were calculated by the Friedewald formula (7). In the diabetic population, glycated hemoglobin (HbA_{1c}) levels were measured by a colorimetric manuel method with a commercial kit (Merck Biotrol). The upper limit of normality was < 7.2%. Urinary microalbumin was measured by Micral-Test II (Boehringer Mannheim), from spot urine samples collected early in the morning and was calculated by estimating the albumin concentration per milliliter of urine.

Assay of plasma lipid hydroperoxide (LHP) levels. Plasma LHP levels were determined spectrophotometrically according to the method of ferrous oxidation with xylenol orange (FOX 1) (22). Hydroperoxides oxidize ferrous to ferric ions selectively in dilute acid and the resultant ferric ions can be determined by using ferric-sensitive dyes as an indirect measure of hydroperoxide concentration. Xylenol orange binds ferric ions with high selectivity to produce a coloured (blue-purple) complex with an extinction coefficient of $1.5 \times 10^4\ M^{-1} \times cm^{-1}$.

Briefly, 50 µl of test sample was added to 950 µl of FOX 1 reagent, vortexed, and incubated at room temperature for a minimum of 30 min, at which time color development was virtually complete. The absorbance was read at 560 nm after removal of any flocculated material by centrifugation. The signal was read against a H_2O_2 standard curve.

Statistics. Computer program InStat was used for data analysis. All results concerning type 1 diabetics and their age-matched controls were expressed as arithmetic mean ± standard deviation (± S. D.). All data were not normally distributed. Three groups of diabetic patients were compared to each other using Kruskal-Wallis nonparametric ANOVA. On the other hand, diabetic patients were compared to their appropriate controls using Mann-Whitney *U*-test. Spearman's rank correlation coefficients were used to investigate various oxidative stress parameters. When not specified $p < 0.05$ was considered significant.

RESULTS

LHP levels and lipid profile of early stage type 1 diabetic patients and their controls are given in Table 2. LHP levels of prepubertal diabetic patients and their controls were not different ($p > 0.05$). LHP levels were increased in adolescent ($p < 0.01$) and young adult ($p < 0.01$) early stage type 1 diabetic patients and their controls. LHP and HbA_{1c} levels were significantly correlated in adolescent ($r = 0.77$, $p < 0.05$) and young adult diabetic patients ($r = 0.53$, $p < 0.05$). There was no correlation between plasma LHP levels and the lipid profile parameters except triglycerides ($r = 0.83$, $p < 0.05$) in early stage type 1 diabetic patients.

Lipid profile parameters of three groups of diabetic patients were compared by Kruskal-Wallis nonparametric ANOVA test and were not found to be statistically different from each other ($p > 0.05$). On the other hand, lipid profile parameters of diabetic patients were compared with their appropriate controls using Mann-Whitney *U*-test, and no difference was found except HDL and apolipoprotein A-1 levels (Table 2).

DISCUSSION

The plasma lipid peroxidation products in normolipidemic diabetics are probably the result of two main sources: (a) oxidation of biomembrane lipids and (b) oxidation of circulating lipoproteins such as LDL supporting the association between oxidative modification of lipids and atherogenesis in diabetes (6).

In our study we found significant augmentations in LHP values in type 1 diabetics without elevated serum total cholesterol and triglycerides levels. This observation supports the hypothesis of a role of oxygen reactive species in diabetes independently of lipid serum content. LHP are a large family of the first by-products of oxidized lipids, and their quantification could become a useful biomarker (1). TBARs (9, 10, 11, 13, 15) and MDA (5, 12, 23) were used as markers of lipid peroxidation in all studies concerning type 1 diabetes, however, in our study, we measured total LHP levels which were known not to suffer from extraction losses (3). On

Table 2 *Lipid hydroperoxide levels and lipid profile in early stage type 1 diabetic patients and their controls*

	Prepubertal diabetic group (n=12)	Prepubertal control group (n=14)	Adolescent diabetic group (n=18)	Adolescent control group (n=15)	Young adult diabetic group (n=21)	Young adult control group (n=19)
LHP (μmol/l)	1.44 ± 0.29	1.04 ± 0.50	2.10 ± 1.09**	1.00 ± 0.42	1.96 ± 0.78**	1.15 ± 0.41
Triglycerides (mmol/l)	1.26 ± 0.19	1.64 ± 0.77	1.12 ± 0.79	1.13 ± 0.41	1.24 ± 0.76	0.97 ± 0.43
Total cholesterol (mmol/l)	3.75 ± 0.87	3.59 ± 0.76	3.92 ± 0.71	3.87 ± 1.04	4.32 ± 0.64	3.98 ± 0.69
HDL-cholesterol (mmol/l)	1.25 ± 0.33*	1.06 ± 0.40	1.24 ± 0.31*	1.01 ± 0.17	1.23 ± 0.30	1.06 ± 0.25
LDL-cholesterol (mmol/l)	2.10 ± 0.73	1.99 ± 0.76	2.23 ± 0.57	2.33 ± 0.97	2.52 ± 0.54	2.46 ± 0.66
VLDL-cholesterol (mmol/l)	0.50 ± 0.13	0.68 ± 0.37	0.51 ± 0.36	0.57 ± 0.31	0.57 ± 0.35	0.46 ± 0.25
Apolipoprotein A-1 (mg/dl)	99.13 ± 11.42*	88.91 ± 10.49	93.87 ± 11.22*	88.16 ± 9.22	94.65 ± 18.99	92.18 ± 16.03
Apolipoprotein B (mg/dl)	69.85 ± 26.16	59.98 ± 16.49	66.85 ± 18.71	66.63 ± 21.14	68.28 ± 17.19	68.04 ± 18.29
Lipoprotein (a) (mg/dl)	22.95 ± 6.52	22.75 ± 7.83	26.81 ± 6.77	25.28 ± 6.47	20.55 ± 5.15	27.74 ± 4.57

Values are represented as arithmetic mean ± S. D.
*$p < 0.05$, **$p < 0.01$
LHP, lipid hydroperoxides: HDL, high density lipoprotein: LDL, low density lipoprotein: VLDL, very low density lipoprotein.

the other hand, spectrophotometric analysis of TBARs overestimates MDA content, since dialdehydes other than MDA and other plasma components react with thiobarbituric acid to form colored complexes; in fact, the limited specifity of this method is the main reason for questioning the validity of TBARs in evaluating the presence of oxidative stress (5). Our study supports the hypothesis that hyperglycemia is an important factor in generation of increased LHP levels in early-stage type 1 diabetic patients.

In a study with type 1 diabetic patients with and without vascular complications, Ruiz *et al*. (15) had reported that plasma LHP levels were significantly increased compared to their controls. In addition, they had found that LHP levels were significantly correlated with total cholesterol, triglyceride, and HbA_{1c} levels. However, apolipoprotein A-1, apolipoprotein B, and lipoprotein (a) levels were not determined in this study. In another study, Berg *et al*. (3) had found a significant correlation between plasma LHP and HbA_{1c} levels, but apolipoprotein A-1, apolipoprotein B, and lipoprotein (a) levels were not measured in this study. In a study concerning type 1 diabetic children and adolescents, Torres *et al*. (19) had determined total cholesterol, HDL-cholesterol, apolipoprotein A-1, apolipoprotein B, lipoprotein (a) levels. They had found that HDL-cholesterol, and apolipoprotein A-1 levels of diabetic patients were significantly increased compared to their controls, but lipoprotein (a) levels were not different. However, they had not determined LHP levels. In our study, we found that apolipoprotein B and lipoprotein (a) levels of three groups of diabetic patients were not different from their controls. We also found that HDL-cholesterol and apolipoprotein A-1 levels of prepubertal and adolescent diabetic patients were significantly increased compared to their controls.

Serum total cholesterol and triglycerides levels of three groups of early stage type 1 diabetic patients were not different compared to their controls. However, LHP levels were increased significantly in adolescent and young adult early stage type 1 diabetic patients compared to their controls. The significant increase in LHP levels in these two groups of patients was independent from their lipid profile parameters. Our study has provided evidence which demonstrates that glycemic control is associated with plasma LHP levels in adolescent and young adult early stage type 1 diabetic patients. From this study we can not conclude whether increased lipid peroxidation is a cause or a secondary process in diabetes. Even if lipid peroxidation is not a primer in diabetic complications it certainly contributes to its progression. The antioxidant approaches of early stage type 1 diabetic patients, even if they are normolipidemic, should be considered in future preventive and therapeutic strategies. The lack of difference in LHP levels of prepubertal diabetic patients and their controls might be explained by the intense hormonal and metabolic changes during puberty. We believe that further studies are required to clarify the effects of hormonal and metabolic changes on LHP levels in adolescents.

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