

Plasma lipoprotein(a) concentration and phenotypes in diabetes mellitus

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Summary. Patients with Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus are at increased risk of developing atherosclerotic vascular diseases. A variety of lipoprotein abnormalities have been described as being associated with this increased risk. In this study, apo(a) isoform frequencies and lipoprotein(a) [Lp(a)] concentrations were determined in Type 1 and Type 2 diabetic patients in order to investigate a possible contribution of Lp(a) to the increased risk for atherosclerosis in diabetes. No significant differences in plasma Lp(a) concentrations were found in two ethnically different populations (Austrians from the province of Tyrol and Hungarians from Budapest) in either type of diabetes when compared to respective con-

trol groups (91 Type 1 and 112 Type 2 diabetic patients vs 202 control subjects in the Hungarian study and 44 Type 1 diabetic and 44 Type 2 diabetic vs 125 control subjects in the Austrian study). There were also no significant apo(a) isoform frequency differences between both patient groups and control subjects in the two study groups. These data, obtained from two large ethnically different populations, provide no evidence of a contribution of Lp(a) to the increased risk for atherosclerosis in diabetes.

Key words: Diabetes mellitus, lipoprotein(a), apo(a) isoforms.

The well-known high rate of cardiovascular morbidity and mortality in diabetic patients indicates the existence of risk factors related to the disease, which may enhance the susceptibility to atherosclerosis [1]. Several studies indicate that lipoprotein alterations found in long-term diabetes mellitus contribute to the development of late macrovascular atherosclerotic complications. Careful analysis of lipoprotein metabolism did not reveal any pathological changes specific for diabetes. Poor metabolic control per se does not fully explain the lipoprotein abnormalities in all types of diabetic patients [2, 3]. It has been postulated that diabetic dyslipidaemia contributes to the atherosclerotic process in the same way as in other, non-diabetic patients [4].

In this study we investigated whether diabetes has an effect on the atherogenic lipoprotein(a) (Lp(a)). Numerous case-control studies have provided evidence for the significance of elevated Lp(a) plasma concentrations as a risk factor for atherosclerotic diseases [5–7]. Therefore, diabetes-induced elevations of Lp(a) might contribute to the increased risk for coronary heart disease in the diabetic population. The atherogenicity of Lp(a) may be partly explained by its high affinity to glycosaminoglycans of the extracellular matrix [8], or alternatively, by

its ability to interfere in vitro with the fibrinolytic cascade [9].

Lp(a) is a low-density lipoprotein (LDL)-like particle which carries the Lp(a)-specific glycoprotein apo(a) [10–12]. Sequence comparisons as well as immunochemical investigations have demonstrated the high structural similarity between apo(a) and plasminogen [13–15]. Apo(a) exhibits a genetically determined size polymorphism. Six different genetic isoforms (designated F, B, S₁, S₂, S₃ and S₄ according to different electrophoretic mobilities), that vary in size from about 400 kD to more than 800 kDa were originally discovered [16]. Meanwhile, improved techniques at higher resolution have revealed more than 20 protein isoforms [17]. Quantitative Southern blotting and pulsed-field gel electrophoresis have shown that this polymorphism results from varying numbers of tandem kringle 4 repeats, leading to more than 19 different apo(a) alleles [18–21]. In the general population, the size of these isoforms is inversely related to the plasma concentration of Lp(a). Large isoforms are associated with low Lp(a) and small isoforms with high Lp(a) concentrations in plasma. In healthy Caucasians, roughly half of the variability in Lp(a) levels is attributed to the apo(a) size polymorphism [22].

Table 1. Lipid and lipoprotein plasma concentrations, laboratory data and clinical characterization (mean \pm SD) of Hungarian subjects

	Type 1 diabetic patients <i>n</i> = 91	Type 2 diabetic patients <i>n</i> = 112	Control subjects <i>n</i> = 202
Cholesterol (mmol/l)	5.7 \pm 2.0	5.8 \pm 1.3	5.6 \pm 1.1
Triglycerides (mmol/l)	2.3 \pm 4.0	2.6 \pm 2.0	– –
HDL cholesterol (mmol/l)	1.3 \pm 0.5 ^a	1.0 \pm 0.3 ^a	1.2 \pm 0.4
HDL ₂ cholesterol (mmol/l)	0.3 \pm 0.2 ^a	0.2 \pm 0.1 ^a	0.3 \pm 0.1 ^a
HDL ₃ cholesterol (mmol/l)	1.0 \pm 0.4 ^a	0.8 \pm 0.3 ^a	0.8 \pm 0.2 ^a
LDL cholesterol (mmol/l)	3.9 \pm 1.5	4.2 \pm 1.2	– –
Lp(a) (mean \pm SD) (mg/dl)	12.1 \pm 20.6	12.2 \pm 17.9	10.5 \pm 13.5
Lp(a) (median) (mg/dl)	3.8	5.2	5.7
Lp(a) (quartiles: 25th, 75th)	1.4 10.5	1.5 11.9	2.0 12.5
Age of all subjects (years)	41.5 \pm 15.1 ^a	60.5 \pm 12.2 ^a	37.4 \pm 9.4 ^a
Age of males (years)	37.3 \pm 13.8	60.4 \pm 12.9	36.6 \pm 8.9
Age of females (years)	45.8 \pm 15.2	60.8 \pm 11.4	39.2 \pm 10.3
BMI (kg/m ²)	23.4 \pm 3.6 ^a	27.6 \pm 4.3 ^a	– –
Duration of diabetes (years)	11.9 \pm 11.2 ^a	7.8 \pm 6.7 ^a	– –
Glucose (mmol/l)	12.3 \pm 4.7 ^a	9.5 \pm 3.7 ^a	– –
HbA _{1c} (%)	7.4 \pm 1.9	7.3 \pm 1.8	– –
Fructosamine (mmol/l)	2.6 \pm 0.5	2.5 \pm 1.2	– –
β -2-microglobulin (mg/l)	1.8 \pm 1.5	2.6 \pm 1.6	– –

^a $p < 0.001$ (ANOVA)

A variety of other genetic and non-genetic factors beside the size variation at the apo(a) gene locus have been described which also effect plasma Lp(a) levels. These include defective alleles at the LDL-receptor gene [23], acute-phase reactions [24], or hormones [25]. Lp(a) levels may also be significantly elevated secondary to diseases with known increased risk for atherosclerosis (e.g. renal diseases) and might therefore in part explain this increased risk.

In order to investigate the role of Lp(a) as a possible risk factor for atherosclerotic complications in diabetes, we measured Lp(a) plasma concentrations and apo(a) phenotype frequencies in two ethnically different European populations of diabetic patients and compared them with a control group.

Subjects, materials and methods

The study populations consisted of 135 patients with Type 1 (insulin-dependent) diabetes receiving insulin therapy and 156 patients with Type 2 (non-insulin-dependent) diabetes with no insulin treatment. All patients were free of liver and renal diseases as ascertained by routine measurement of liver enzymes (GOT, GPT, γ GT), creatinine and urea in plasma. However, patients urine was not checked for microalbuminuria. Fasting EDTA blood samples from patients were obtained from three out-patient clinics (one in Innsbruck, two in Budapest). Non-fasting blood samples were randomly obtained from blood donors at the blood banks in Innsbruck (Austria) and Budapest (Hungary). Plasma was obtained by low-speed centrifugation and stored at -20°C for a maximum of 6 months until further use. The plasma samples from Hungarian patients and control subjects were transported to Innsbruck on dry ice.

Hungarian group

Table 1 lists the characteristics of the Hungarian diabetic patients and the control group. The sex ratio was about 1:1 for diabetic patients, whereas the control group contained about two times as many men. According to disease type, the age of patients with Type 1 diabetes was significantly lower as compared to Type 2 diabetes patients.

Type 2 diabetic patients had higher body weight and their duration of diabetes was shorter (Table 1). Concentrations of haemoglobin A_{1c} and fructosamine were identical in both diabetic groups (Table 1). Concentration of total HDL cholesterol and HDL₂ fraction were significantly lower in Type 2 diabetic patients, whereas the concentration of HDL₃ cholesterol in Type 1 patients was higher than in Type 2 and control groups (Table 1).

Austrian group

The clinical properties and glycaemic control of diabetic patients from Austria were comparable to those of the Hungarian patients (Table 2). The only difference was in the lipid profile: total cholesterol concentration was lower in subjects with Type 1 diabetes than in the control and Type 2 diabetes groups (Table 2). The lower plasma levels of apo AI in subjects with Type 2 diabetes corresponded with the results of HDL cholesterol concentration, which were determined only in the Hungarian diabetic patients.

Plasma concentrations of Lp(a) were determined using an ELISA exactly as previously described [26]. The "Norm Control Lp(a) Human" from Immuno AG (Vienna, Austria) served as secondary standard. Apo(a) phenotyping was performed by SDS-PAGE of whole plasma samples followed by immunoblotting according to Utermann et al. [16] with some modifications [22]. Haemoglobin A_{1c} was measured by microcolumn chromatography, whereas fructosamine was estimated by colorimetric assay. Fasting glucose was evaluated from plasma by the glucose oxidase method and total cholesterol was analysed with a commercially available kit (Boehringer Mannheim, Mannheim, FRG). Cholesterol concentrations in HDL and its subfractions HDL₂ and HDL₃ were measured by a dual-precipitation method according to Patsch et al. [27]. LDL cholesterol was calculated using Friedewald's formula [28]. Apo AI and apo B were assayed by an immunonephelometric assay (Tinaquant, Boehringer Mannheim). β -2-microglobulin was estimated with a commercially available kit from Pharmacia, Uppsala, Sweden.

Statistical analysis

Multiple linear regression was used to adjust total cholesterol, HDL cholesterol and apolipoprotein levels for the linear effects of age and sex. The equality of Lp(a) levels among diabetic and control groups was tested by the non-parametric Kruskal-Wallis test. Other

Table 2. Lipid and lipoprotein plasma concentrations, laboratory data and clinical characterization (mean \pm SD) of Austrian subjects

	Type 1 diabetic patients <i>n</i> = 44	Type 2 diabetic patients <i>n</i> = 44	Control subjects <i>n</i> = 125
Cholesterol (mmol/l)	4.9 \pm 1.0 ^a	5.8 \pm 1.5 ^a	5.6 \pm 1.1
Triglycerides (mmol/l)	1.2 \pm 0.6	3.6 \pm 5.6	– –
HDL cholesterol (mmol/l)	1.7 \pm 0.5 ^a	1.3 \pm 0.4 ^a	– –
Apo A1 (mg/dl)	163 \pm 37 ^a	149 \pm 34 ^a	157 \pm 28
Apo B (mg/dl)	70 \pm 23	88 \pm 24	64 \pm 13
LDL cholesterol (mmol/l)	2.7 \pm 0.9	3.4 \pm 0.8	– –
Lp(a) (mg/dl)	10.8 \pm 16.6	14.6 \pm 21.0	12.2 \pm 16.5
Lp(a) (median) (mg/dl)	4.6	4.6	6.2
Lp(a) (quartiles: 25th, 75th)	1.6 12.7	1.8 14.8	2.5 14.8
Age of all subjects (years)	38.5 \pm 13.6 ^a	61.8 \pm 11.4 ^a	37.6 \pm 10.4 ^a
Age of males (years)	39.1 \pm 13.3	60.0 \pm 11.5	38.6 \pm 9.5
Age of females (years)	36.8 \pm 14.6	63.9 \pm 11.1	36.3 \pm 10.9
BMI (kg/m ²)	23.2 \pm 2.9 ^a	26.8 \pm 4.8 ^a	– –
Duration of diabetes (years)	12.9 \pm 9.2 ^a	10.0 \pm 7.2 ^a	– –
Glucose (mmol/l)	10.1 \pm 5.1	9.8 \pm 3.5	– –
HbA _{1c} (%)	7.9 \pm 1.8	7.7 \pm 1.5	– –

^a *p* < 0.001 (ANOVA)

Table 3. Frequencies of apo(a) phenotypes and Lp(a) concentrations in Hungarian diabetic patients and control subjects

Phenotypes	Type 1 diabetic patients			Type 2 diabetic patients			Control subjects		
	<i>n</i>	%	mean \pm SD	<i>n</i>	%	mean \pm SD	<i>n</i>	%	mean \pm SD
B	1	1.1	59.0	1	0.9	27.0	2	1.0	28.5 \pm 7.0
S1	1	1.1	6.6	1	0.9	10.1	4	2.0	23.0 \pm 13.8
S2	16	17.6	35.3 \pm 34.6	17	15.2	23.5 \pm 23.4	36	17.8	19.5 \pm 22.0
S3	18	19.8	7.7 \pm 9.6	18	16.1	5.7 \pm 5.3	55	27.2	8.9 \pm 10.2
S4	18	19.8	4.6 \pm 5.4	25	22.3	4.4 \pm 4.7	54	26.7	6.1 \pm 5.5
0	12	13.2	1.1 \pm 0.9	17	15.2	1.8 \pm 1.8	14	6.9	1.1 \pm 1.0
S1S2	1	1.1	1.2	1	0.9	34.0	2	1.0	24.0 \pm 22.6
S2S3	4	4.4	30.6 \pm 22.6	5	4.5	5.8 \pm 4.6	9	4.5	8.2 \pm 9.6
S2S4	8	8.8	3.8 \pm 2.4	9	8.0	36.9 \pm 27.9	9	4.5	20.8 \pm 13.6
S3S4	12	13.2	– –	18	16.1	16.0 \pm 19.6	16	7.9	5.8 \pm 4.2
BS4	–	–	– –	1	0.9	38.5	1	0.5	31.0
Total	91	100	12.1 \pm 20.6	112	100	12.2 \pm 17.9	202	100	10.5 \pm 13.5

multiple group means were compared by analysis of variance (ANOVA). For the calculation of effects of apo(a) phenotypes on Lp(a) concentrations (R^2 statistics) the values for Lp(a) concentrations were log-transformed prior to ANOVA. Pearson's chi-square statistics was used to test the independence of the apo(a) type frequencies between the diabetic patients and the control subjects. The Statistical Package for Social Sciences (SPSS) was used.

Results

Lp(a) concentrations and apo(a) phenotype distribution

The mean Lp(a) concentrations in both Type 1 and Type 2 diabetic patients did not differ from those of control subjects in either the Hungarian or Austrian groups (Tables 1 and 2). Lp(a) concentration showed no significant correlation with other lipid or glycaemic parameters, including the level of β -2-microglobulin. The apo(a) phenotype frequencies are shown in Tables 3 (Hungarian) and 4 (Austrian). No significant difference in distribution was observed in the two diabetic or control groups. Average Lp(a) levels for each phenotype in diabetic and control subjects corresponding to the two populations are shown in detail in Tables 3 and 4. If the Hungarian and Austrian patients were pooled within their respective study groups,

the mean Lp(a) concentration was 11.2 \pm 18.3 mg/dl in Type 1 patients, 13.1 \pm 19.2 mg/dl in Type 2 patients and 11.4 \pm 14.8 mg/dl in control subjects ($p = 0.54$, NS). When the apo(a) frequency distribution was calculated in these pooled groups, no significant difference was obtained as well ($p = 0.26298$). Consistent with the previously shown inverse relationship between the molecular weight of apo(a) isoforms and Lp(a) levels, the effects of apo(a) alleles on Lp(a) levels were comparable in the Hungarian patients and control subjects (R^2 values: 0.24, $p < 0.001$ (control subjects); 0.41, $p < 0.001$ (Type 1 diabetic patients); 0.36, $p < 0.001$ (Type 2 diabetic patients)).

Discussion

Conflicting data for Lp(a) levels in diabetic patients exist in the literature. One of the earliest studies in this field did not reveal a significant difference in Lp(a) levels between diabetic patients (not differentiated for Types 1 and 2) and normal control subjects [29]. Mertz and Bürvenich [30] then reported high Lp(a) concentrations in diabetic patients, which were confirmed by others for diabetic patients with established nephropathy [31]. More recently, normal Lp(a) levels were reported in

Table 4. Frequencies of apo(a) phenotypes and Lp(a) concentrations in Austrian diabetic patients and control subjects

Phenotypes	Type 1 diabetic patients			Type 2 diabetic patients			Control subjects		
	<i>n</i>	%	mean ± SD	<i>n</i>	%	mean ± SD	<i>n</i>	%	mean ± SD
B	—	—	—	—	—	—	2	1.6	30.1 ± 35.2
S1	—	—	—	1	2.2	3.7	2	1.6	32.5 ± 0.7
S2	5	11.3	17.3 ± 16.9	4	9.1	15.4 ± 15.8	20	16.1	23.1 ± 21.2
S3	17	38.6	4.4 ± 4.7	10	22.7	9.3 ± 8.9	32	25.8	7.3 ± 9.6
S4	11	25.0	12.4 ± 15.4	12	27.3	4.4 ± 3.3	33	26.6	5.0 ± 4.4
0	4	9.1	0.8 ± 0.7	5	11.4	1.0 ± 1.0	4	3.2	1.6 ± 0.9
S1S2	—	—	—	—	—	—	1	0.8	0.9
S1S3	—	—	—	1	2.3	4.4	1	0.8	25.0
S1S4	1	2.3	2.2	—	—	—	1	0.8	4.0
S2S3	3	6.8	9.1 ± 4.2	1	2.3	58.0	10	8.1	25.3 ± 28.1
S2S4	2	4.6	57.5 ± 43.1	5	11.3	46.7 ± 43.2	9	7.3	14.9 ± 12.6
S3S4	1	2.3	31.0	4	9.1	22.2 ± 16.8	9	7.3	12.9 ± 10.5
BS3	—	—	—	1	2.3	42.0	—	—	—
Total	44	100	10.8 ± 16.6	44	100	14.6 ± 21.8	124	100	12.2 ± 16.5

45 subjects with Type 2 diabetes [32] as well as in black children and white children with Type 1 diabetes [33]. In a small number of poorly-controlled Type 1 patients, a decline in Lp(a) was observed with improved glycaemic control [34]. In a similar study, Lp(a) levels in 12 Type 1 subjects also declined with metabolic improvement. However, the authors could not observe a correlation between the changes in glycated haemoglobin and Lp(a) concentration [35]. The same authors recently did not observe a change in Lp(a) concentrations with improved glycaemic control in Type 2 diabetic patients [36]. Increased Lp(a) values which positively correlated with HbA_{1c} levels were reported in ten poorly-controlled diabetic white children when compared to a control group. In contrast, no correlation of Lp(a) concentrations with the glycaemic status was found in the same study in black children with Type 1 diabetes [33].

In nephrotic renal disease, a correlation was reported between albuminuria and raised Lp(a) concentrations [37]. Significantly positive correlations between Lp(a) and urinary albumin concentrations were also shown in diabetic patients, who were not characterized in detail [31]. Elevated levels of Lp(a) in patients with Type 1 diabetes combined with urinary protein losses were reported by Jenkins et al. [38]. In a later study, Type 1 diabetic patients with microalbuminuria had significantly higher Lp(a) concentrations than those without microalbuminuria of the same sex, age, duration of diabetes and haemoglobin A_{1c} level [39]. However, a similar study failed to confirm these observations [40].

In our study the large groups of patients with both Types 1 and 2 diabetes were well controlled and Lp(a) concentrations were independent of glycated haemoglobin, fructosamine, glucose level and other lipoprotein concentrations. Most previous reports have focused on a much smaller number of patients than the present investigation. Furthermore, no apo(a) isoform analysis was performed. In small study groups, chance variation in apo(a) isoform frequencies may well account for differences between diabetic patients and control subjects.

We found no significant differences in Lp(a) levels among well-controlled patients with Type 1 and Type 2

diabetes and normal volunteers in two ethnically different populations. There were no differences in apo(a) phenotype frequencies between diabetic patients in our study. Thus, diabetic patients and control subjects were essentially controlled for any potential differences in Lp(a) concentration due to apo(a) allele frequency differences. Differences in the sex ratio between the Hungarian control and diabetic subjects are unlikely to have biased our results. Gender has no or only a moderate effect on Lp(a). Moreover, no significant gender difference was present in the Austrian sample. We therefore conclude that mechanisms independent of genetic apo(a) types and Lp(a) levels are responsible for the development of diabetic vascular complications. One additional finding of our study was that the effects of apo(a) phenotypes on Lp(a) levels are similar in diabetic patients and control subjects. The differences in age and sex distribution have no significance for this study since Lp(a) concentrations were reported to be independent of age and sex [41].

We also measured the plasma level of β -2-microglobulin as a marker for proteinuria and impaired renal function and could not find an association between Lp(a) and β -2-microglobulin plasma concentration in the two diabetic groups.

Atherosclerosis is the main cause of mortality in diabetic patients [1, 3]. Lipid abnormalities and their pathophysiology in diabetes are not exactly clear [2]. One important factor contributing to atherosclerotic vascular disease in diabetes was considered to be Lp(a). In this study we demonstrate that the Lp(a) levels and apo(a) phenotype frequencies in Type 1 and Type 2 patients do not differ from those in normal control subjects from two Caucasian populations. These results suggest a rather unlikely role of Lp(a) as a risk factor contributing to atherosclerotic complications in diabetes.

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