Serum Lipoprotein(a) in Patients With Diabetes Mellitus

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OBJECTIVE — To investigate subjects with different types of diabetes mellitus regarding their serum levels of lipoprotein(a). High serum Lp(a) concentration is associated with a high risk of coronary heart disease. Diabetic patients are prone to developing coronary heart disease.

RESEARCH DESIGN AND METHODS — The subjects were 66 type I diabetic patients, 100 type II diabetic patients treated with diet alone or diet combined with oral hypoglycemic agents, and 46 insulin-requiring type II diabetic patients. Subjects were compared with 142 nondiabetic outpatients.

RESULTS — Subjects with insulin-requiring type II diabetes mellitus were found to have an increase both in serum Lp(a) concentration and in prevalence of serum Lp(a) concentration >30 mg/dl compared with the other groups of diabetic patients and nondiabetic control subjects. A nonsignificant increase in the prevalence of coronary heart disease was also found in insulin-requiring type II diabetic patients. The levels of serum concentrations of Lp(a) were not significantly related to the degree of glycemic control, duration of diabetes, presence of macrovascular disease, or intake of female hormone therapy. High levels of Lp(a) in this group of diabetic patients could not be explained by the presence of albuminuria.

CONCLUSIONS — Insulin-requiring type II diabetic patients have high levels of Lp(a). Chronic hyperinsulinemia might be an eventual causal factor.

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Lp(a), LIPOPROTEIN(a); TYPE I DIABETES, INSULIN-DEPENDENT DIABETES MELLITUS; TYPE II DIABETES, NON-INSULIN-DEPENDENT DIABETES MELLITUS; BMI, BODY MASS INDEX; HLA, HUMAN LEUKOCYTE ANTIGEN; CHD, CORONARY HEART DISEASE; BP, BLOOD PRESSURE; WHO, WORLD HEALTH ORGANIZATION; HDL, HIGH-DENSITY LIPOPROTEIN; LDL, LOW-DENSITY LIPOPROTEIN; ELISA, ENZYME-LINKED IMMUNOSORBENT ASSAY; HPLC, HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY; CV, COEFFICIENT OF VARIATION; RIA, RADIOIMMUNOASSAY; ANOVA, ANALYSIS OF VARIANCE; WESDR, WISCONSIN EPIDEMIOLOGY STUDY OF DIABETIC RETINOPATHY.

Serum Lp(a) concentrations >30 mg/dl are considered a risk factor for CHD, particularly when associated with hypercholesterolemia (1,2). Diabetes confers an increased risk for CHD (3). Data on the relationship between Lp(a) and diabetes are scarce. Most studies have focused on the influence of the degree of glycemic control on serum Lp(a) concentrations (4–7). The purpose of our study was to measure the serum concentrations of Lp(a) in patients with diabetes and to analyze the results according to the type of diabetes, the mode of treatment, the degree of glycemic control, and the absence or presence of CHD.

RESEARCH DESIGN AND METHODS

— Between January and July 1990, 212 consecutive diabetic patients were studied while attending the diabetic clinics in the Hospital of Jolimont and the University Clinic of Mont-Godinne. They were divided into three groups: type I diabetic patients (group 1; $n = 66$); type II diabetic patients, treated with diet alone or diet combined with oral hypoglycemic agents (group 2; $n = 100$); and insulin-requiring type II diabetic patients (group 3; $n = 46$). The classification of type I and II diabetic patients was based on clinical and biological criteria from different studies (8–10). Type I diabetic patients were characterized by the brutal onset of their symptoms of diabetes; they were totally insulin dependent and ketosis prone, as assessed by C-peptide levels <0.1 pM in the basal state and after a standardized meal. BMI was normal (median, 24 kg/m^2). In contrast, type II diabetic patients were characterized by the insidious onset of the disease and the absence of ketosis. They all had a residual insulin secretion as shown by C-peptide levels >0.1 pM. These patients were overweight (BMI, median 30 kg/m^2). The classification of type I and II diabetes was not based on the HLA DR3-DR4 antigen determination or on the presence of islet cell anti-

bodies, because these parameters were not routinely assessed in our type II diabetic patients. Age (mean ± SD) was 45 ± 18 yr for group 1 (median 45 yr) and 65 ± 9 yr for groups 2 (median 64 yr) and 3 (median 67 yr). The known duration of diabetes was not significantly different in group 1 (13 ± 11 yr) and group 3 (13 ± 9 yr) but was lower in group 2 than in the two other groups (9 ± 8 yr; $P < 0.001$ vs. group 1 and $P = 0.007$ vs. group 3). The sex ratio (males/females) was 36/30, 38/62, and 12/34 for group 1, 2, and 3, respectively (NS).

CHD was evaluated in all patients on the basis of clinical data, electrocardiographic abnormalities, and/or positive coronary angiography. A subset of 131 patients (34 in group 1, 63 in group 2, 34 in group 3), in whom at least two successive BP measurements were available and/or who were treated with antihypertensive drugs, was considered for the assessment of hypertension. Patients were considered hypertensive if their BP levels were >160/95 mmHg, according to WHO criteria, and/or if they received antihypertensive treatment. Microalbuminuria and macroproteinuria were determined in 89 patients (30 in group 1, 39 in group 2, 20 in group 3). Information concerning the use of hormonal therapy was available in 104 of 126 female diabetic patients. Thirteen (13%) were found to receive such a treatment (hormone replacement therapy in 12 cases and tamoxifen for endometriosis in 1 case). No patients were taking other drugs (e.g., thiazide diuretics, β-blockers, hypolipidemic drugs) known to modify serum lipoproteins or Lp(a) levels.

A control population of 142 nondiabetic outpatients (58 ± 18 yr; median 63; sex ratio 79/63) was also studied. No attempt was made to match diabetic patients with control subjects for age or sex, because Lp(a) levels have been shown not to be influenced by these parameters (11).

After an overnight fast, blood samples were collected on the same day for measurements of Lp(a), total and HDL cholesterol, triglycerides, and HbA_{1c}. The serum Lp(a) concentration was measured by ELISA immunoassay (Tintelize Lp(a), Biopool, Umea, Sweden) on samples refrigerated for 3 days or on frozen serum stored at −25°C for up to 2 wk. No samples were thawed more than once. The intra-assay CV was 6.9% at 7.0 mg/dl ($n = 7$), 2.4% at 28 mg/dl ($n = 9$), and 5.6% at 54 mg/dl ($n = 11$), and the interassay CV was 5.7% at 23 mg/dl ($n = 27$). Enzymatic techniques were used to measure total cholesterol (CHOD-PAP, Merck, Darmstadt, Germany) and triglycerides (GPO-PAP, Merck). HDL cholesterol was determined by assaying total cholesterol concentration in the supernatant obtained after precipitation of lipoprotein of density lower than HDL by a mixture of phosphotungstic acid and magnesium chloride (12). LDL cholesterol was calculated following the Friedewald's formula (13). C-peptide was measured by RIA with a commercially available kit (C-PEP/RIA/CT, Medgenix, Fleurus, Belgium). HbA_{1c} was assayed by HPLC on an AUTO A_{1C} chipped with a column packed with Micropearl SF-W-A_{1C} as stationary phase (Dic Kyoto Daiichi, Menarini, Brussels). At an HbA_{1c} value of 3.75%, the intra-assay CV was 3.5% ($n = 19$) and the interassay CV was 5.2% ($n = 15$). Microalbuminuria was assayed by RIA (Albumine-RIA, Pharmacia, Uppsala, Sweden) and was considered present when albumin levels were between 30 and 300 mg/24 h. The dipstick test was used to detect macroproteinuria.

Homogeneity of categorical variables between groups was studied by X^2 test and, if significant, groups were compared 2-by-2 by χ^2 or Fisher's exact tests when appropriate. For continuous variables, homogeneity between groups was assessed by Kruskal-Wallis ANOVA by ranks. In case of a significant difference, 2-by-2 comparisons were performed with Wilcoxon rank-sum tests. Correlations were studied by Spearman rank-correlation coefficient. All tests were two-tailed. $P < 0.05$ was considered statistically significant.

RESULTS — In the control population, median Lp(a) concentration was 9.3 mg/dl. The prevalence of Lp(a) >30 mg/dl was 20%. As shown in Table 1, neither parameter was significantly different in control subjects and in group 1 and 2 diabetic patients. In contrast, in group 3, Lp(a) was significantly higher than in the control subjects ($P = 0.04$) or the other diabetic groups (19.3 vs. 10.0 mg/dl [group 1; $P = 0.01$] and 8.3 mg/dl [group 2; $P = 0.05$]). The prevalence of Lp(a) concentration >30 mg/dl was also significantly higher in group 3 (39% vs. 20, 20, and 18% in control subjects, group 1, and group 2, respectively). Table 1 shows that total cholesterol, LDL cholesterol, HDL cholesterol, and HbA_{1c} were comparable in the three diabetic groups. However, triglycerides were higher in groups 2 and 3 than in group 1.

In the total group of patients, Lp(a) levels were correlated with total cholesterol ($r_s = 0.166$; $P = 0.018$) and LDL cholesterol ($r_s = 0.178$; $P = 0.013$), but not with triglycerides ($r_s = -0.032$) and HDL cholesterol ($r_s = 0.071$). There was also a trend for a correlation between Lp(a) and HbA_{1c}, but it did not reach the level of significance ($r_s = 0.140$; $P = 0.066$). No correlation was found between Lp(a) and HbA_{1c} when considering the different groups of diabetic subjects separately: group 1, $r_s = 0.156$ (NS); group 2, $r_s = 0.150$ (NS); group 3, $r_s = 0.046$ (NS). No correlation was found between Lp(a) levels and age ($r_s = 0.046$), BMI ($r_s = 0.048$), or duration of diabetes ($r_s = 0.009$).

High BP levels were more frequent in group 2 ($n = 32$; 51%) and group 3 ($n = 21$; 62%) than in group 1 ($n = 8$; 24%; $P = 0.009$ vs. group 2 and $P = 0.001$ vs. group 3). CHD was slightly but not significantly more frequent in group 3 ($n = 13$; 28%) than in group 1 ($n = 13$; 20%) and in group 2

Table 1—*Lp(a), cholesterol, triglycerides, and HbA_{1c} levels in nondiabetic control subjects and diabetic patients*

	Study groups			
	Nondiabetic control	Group 1 Type I diabetic (insulin-treated)	Group 2 Type II diabetic (non-insulin-treated)	Group 3 Type II diabetic (insulin-treated)
n	142	66	100	46
Serum Lp(a) (mg/dl)	21.1 ± 30.4 (9.3)	16.8 ± 18.6 (10.0)	16.6 ± 20.4 (8.3)	26.7 ± 25.0 (19.3)*
Prevalence of Lp(a) (%) >30 mg/dl	20	20	18	39†
Total cholesterol (mM)	6.01 ± 1.19 (5.85)	5.56 ± 1.22 (5.43)	6.03 ± 1.17 (6.06)	6.01 ± 1.46 (5.82)
LDL cholesterol (mM)	—	3.68 ± 1.22 (3.76)	4.04 ± 0.98 (3.96)	3.9 ± 1.35 (3.83)
HDL cholesterol (mM)	—	1.14 ± 0.57 (1.0)	1.01 ± 0.31 (0.96)	1.04 ± 0.34 (0.96)
Triglycerides (mM)	—	1.47 ± 0.90 (1.14)	2.10 ± 1.31 (1.91)‡	1.86 ± 0.83 (1.69)‡
HbA_{1c} (%)	4.8 ± 0.6 (4.8)	8.0 ± 1.9 (7.8)	7.9 ± 2.3 (7.7)	8.5 ± 1.8 (8.2)

Data are means ± SD (median).
*Group 3 different from control subjects ($P = 0.04$), group 1 ($P = 0.01$), and group 2 ($P = 0.05$), with all other differences between groups not statistically significant (Wilcoxon rank-sum tests).
†Group 3 different from control subjects ($P = 0.02$), group 1 ($P = 0.04$), and group 2 ($P = 0.01$), with all other differences between groups not statistically significant (Fisher exact tests).
‡Group 2 and group 3 different from group 1 ($P < 0.001$ and $P = 0.003$, respectively) and not different from themselves (Wilcoxon rank-sum tests).

($n = 20$; 20%). The levels of Lp(a) and the prevalence of Lp(a) concentration >30 md/dl were not significantly different in diabetic patients with or without arterial hypertension (24.5 ± 23.7 vs. 18.9 ± 22.1 mg/dl; 34 vs. 21%) and in patients with or without CHD (18.8 ± 18.5 vs. 18.9 ± 22.0 mg/dl; 24 vs. 23%).

Microalbuminuria was present in 27 patients (10 of 30 in group 1, 13 of 39 in group 2, and 4 of 20 in group 3). Macroproteinuria was observed in 25 patients (6 of 30 in group 1, 10 of 39 in group 2, and 9 of 20 in group 3). Classification by the absence or the presence of microalbuminuria and macroproteinuria was independent of the group classification ($P = 0.39$). Thus, subjects in group 3 had no more nephropathy than patients in groups 1 and 2. The Lp(a) levels and the prevalence of Lp(a) >30 mg/dl were significantly higher in patients with macroproteinuria (25.7 ± 19.5 mg/dl and 40%, respectively) than in normoalbuminuric diabetic subjects (12.8 ± 16.7 mg/dl [$P = 0.002$] and 11% [$P = 0.007$]). They were also higher than in patients with microalbuminuria (13.6 ± 17.6 mg/dl [$P = 0.006$] and 15% [$P = 0.041$]). Among the 13 diabetic women receiving hormone therapy, 4 were in group 1, 7 in group 2, and 2 in group 3 (NS).

CONCLUSIONS— Information about the serum Lp(a) concentration in diabetic patients is still limited. Moreover, most previous studies involved type I diabetic subjects (4,6,7). In this report, we studied type I diabetic and insulin-treated and non-insulin-treated type II diabetic subjects. Our data showed that Lp(a) levels were higher in insulin-treated type II diabetic patients than in the other diabetic groups or control subjects.

The reason why high Lp(a) levels were found in the group of insulin-treated type II diabetic patients remains unclear. Age and known duration of diabetes cannot explain the observed differences. Serum concentrations of Lp(a) have been found to correlate with the severity of hyperglycemia in type I diabetic adults (5,6) and in black type I diabetic children (6), but not in type II diabetic adults (5). Improved metabolic control has been shown to decrease the serum Lp(a) levels (4,7). In our study, the HbA_{1c} levels were slightly higher in insulin-treated type II diabetic patients, but the difference did not reach the level of significance. Moreover, although a trend for a correlation between Lp(a) and HbA_{1c} was found in the whole group of diabetic patients, no correlation was observed when the three groups were taken separately, in particular, in group 3. Interestingly, in a work recently published on Lp(a) in diabetic patients, the correlation between Lp(a) and HbA_{1c} was found to be very similar to that found in our study ($r_s = 0.165$ and $r_s = 0.140$, respectively; 14). Considering our data, we believe that glycemic control itself could hardly account for the increased Lp(a) levels in group 3.

The Lp(a) concentrations have also been shown to be elevated in type I diabetic patients with microalbuminuria (15,16). They correlated with urinary albumin concentration (17). Our analysis in 89 patients showed that Lp(a) levels were higher in subjects with macropro-

teinuria than in those with or without microalbuminuria. However, the prevalence of macroproteinuria in our study was not statistically different in the three groups of diabetic patients. Thus, clinical nephropathy could not account for the higher Lp(a) in group 3 than in other groups.

In humans, few drugs have been shown to affect Lp(a) levels, which are mostly genetically controlled. None of our patients were receiving nicotinic acid, the only drug reported to reduce Lp(a) concentration (18). Recently, it was suggested that hormone therapy could also decrease Lp(a) levels in postmenopausal women (19,20). In this study, the proportion of women reported to receive such hormones was small (13%) and not significantly lower in group 3 than in other groups.

The role of insulin in elevated Lp(a) levels remains speculative. Insulin treatment per se cannot play a role because Lp(a) levels were not elevated in type I diabetic patients. On the other hand, in insulin-requiring type II diabetic subjects, both residual endogenous and exogenous insulin could result in high plasma insulin levels. Epidemiological, clinical, and experimental studies have raised issues concerning the connection between hyperinsulinemia and the development of large-vessel disease (21,22). In insulin-treated type II diabetic subjects, fasting free insulin levels have been found to be significantly higher in patients with than without macrovascular disease (22,23). High Lp(a) levels were also considered a risk factor for CHD (1,2). In this study, the prevalence of CHD was slightly, although not significantly, higher in insulin-requiring type II diabetic subjects than in the other groups. Therefore, it could be speculated that hyperinsulinemia could be a potential link between high Lp(a), insulin-requiring type II diabetes, and CHD. In normal subjects, serum Lp(a) concentration is related not with its catabolic but with its synthetic rate (24). No data are yet available concerning the influence of insulin on the hepatic synthesis of Lp(a).

Finally, because serum Lp(a) level is under genetic control and high levels appear to be related to a particular phenotype (25), it would be of interest to analyze the phenotype pattern of Lp(a) in the different types of diabetes.

Recently, in the WESDR, diabetic patients who died of CHD were found to have Lp(a) concentrations similar to those of diabetic subjects who remained alive (26). However, no mention was made of the type of treatment in the group of the older onset diabetic subjects (insulin- or non-insulin-requiring). Moreover, it has been demonstrated that a high level of Lp(a) is a risk factor for CHD when LDL-cholesterol levels are also elevated (2). In the WESDR, the LDL-cholesterol levels were not measured, and the diabetic patients who died of CHD had relatively normal total cholesterol levels. Thus, a high level of Lp(a) could still be a risk factor for CHD in diabetic patients as in nondiabetic patients.

In conclusion, we found high Lp(a) levels in insulin-requiring type II diabetic patients. Further studies are needed to precisely define Lp(a) metabolism in diabetes and its relationship with residual insulin secretion and insulin treatment.

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