Association of elevated lipoprotein(a) levels and coronary heart disease in NIDDM patients. Relationship with apolipoprotein(a) phenotypes

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Summary Non-insulin-dependent diabetes mellitus (NIDDM) is a strong and independent risk factor for coronary heart disease. We assessed the potential relationship between plasma Lp(a) levels, apo(a) phenotypes and coronary heart disease in a population of NIDDM patients. Seventy-one patients with coronary heart disease, who previously have had transmural myocardial infarction, or significant stenosis on coronary angiography, or positive myocardial thallium scintigraphy, or in combination, were compared with 67 patients without coronary heart disease, who tested negatively upon either coronary angiography, myocardial thallium scintigraphy or a maximal exercise test. The prevalence of plasma Lp(a) levels elevated above the threshold for increased cardiovascular risk (> 0.30 g/l) was significantly higher (p = 0.005) in patients with coronary heart disease (33.8 %) compared to the control group (13.4 %). The relative risk (odds ratio) of coronary heart disease among patients with high Lp(a) con-

centrations was 3.1 (95 % confidence interval, 1.31–7.34; p = 0.01). The overall frequency distribution of apo(a) phenotypes differed significantly between the two groups (p = 0.043). However, the frequency of apo(a) isoforms of low apparent molecular mass (≤ 700 kDa) was of borderline significance (p = 0.067) between patients with or without coronary heart disease (29.6 % and 16.4 %, respectively). In this Caucasian population of NIDDM patients, elevated Lp(a) levels were associated with coronary heart disease, an association which was partially accounted for by the higher frequency of apo(a) isoforms of small size. In multivariate analyses, elevated levels of Lp(a) were independently associated with coronary heart disease (odds ratio 3.48, p = 0.0233). [Diabetologia (1994) 37: 585–591]

Key words NIDDM, lipoprotein(a), apo(a) isoforms, coronary heart disease, lipids, lipoproteins, cardiovascular risk factors.

Non-insulin-dependent diabetes mellitus (NIDDM) is a strong and independent risk factor for cardiovascular and coronary heart disease mortality [1]. Established risk factors, including glucose intolerance, arterial hypertension, central obesity, hypercholesterolaemia or hypertriglyceridaemia, have been shown to contrib-

ute to the high prevalence of coronary heart disease in NIDDM [2, 3]. The situation concerning Lp(a), a recently identified, independent cardiovascular risk factor, is less clear.

Lp(a) is a cholesterol-rich, LDL-like particle whose protein moiety contains apo(a) in addition to apoB. Apo(a) shows a strong sequence homology with plasminogen [4] and exhibits a genetic size polymorphism which arises from variation in the number of repetitive structures, termed "kringles" [5]. Indeed, the size of apo(a) isoforms is inversely related to plasma concentrations of Lp(a) [6]. Furthermore, the apo(a) phenotype determines at least 40 % of the inter-individual variation in plasma Lp(a) level [7].

It has been established that high plasma levels of Lp(a), as well as apo(a) phenotypes of low molecular

mass, are major risk factors for coronary heart disease among Caucasian and non-Caucasian populations which differ markedly in coronary heart disease prevalence and cardiovascular risk factor profiles [8–11]. However, not only is there a paucity of data on the potential contribution of Lp(a) to premature cardiovascular disease in NIDDM, but in addition, this question remains controversial. The majority of the studies reported to date have found no difference in Lp(a) levels and in the distribution of apo(a) phenotypes between NIDDM patients and non-diabetic subjects [12, 13]. Moreover, it remains to be established whether Lp(a) concentration represents a cardiovascular risk factor in NIDDM patients. Two previous studies provided negative results [14, 15], but a recent case-control study suggested that Lp(a) was an independent cardiovascular risk factor in NIDDM patients [16]. These contrasting findings may result from the small number of subjects studied, coupled to the lack of statistical power and above all, to the limitation that "control" groups may not have been properly evaluated for asymptomatic coronary heart disease. Indeed, the high frequency of silent myocardial ischaemia in diabetes [17] might lead to underestimation of the true prevalence of coronary heart disease in the "control" groups when diagnosis was effected either by clinical examination, or by a resting electrocardiogram, or both. Non-invasive investigations such as exercise electrocardiography or thallium myocardial scintigraphy are more sensitive in evaluating coronary heart disease in such patients [18, 19]. Given the conflicting data concerning links between Lp(a) and coronary heart disease in NIDDM subjects, the aim of the present study was to assess the relationship between Lp(a) levels, apo(a) phenotypes and coronary heart disease in a Caucasian population of NIDDM patients in whom the coronary circulation had been extensively evaluated.

Subjects and methods

Subjects

Subjects in the present study were 138 consecutive NIDDM Caucasian patients (107 men and 31 women), who routinely attended a tertiary referral center between 1 June 1992 and 28 February 1993 and who either had evidence of coronary heart disease or were free of coronary heart disease. All the diabetic patients included in this study fulfilled World Health Organisation criteria for NIDDM [20]. Patients who agreed to participate in the study gave their informed written consent. The study was approved by the Ethics Committee of the Centre Hospitalo-Universitaire Lariboisière-Saint-Louis. The patients were classified into two groups. The first group consisted of patients with coronary heart disease (CHD$^+$; $n = 71$), and was composed of 31 patients with previously documented transmural myocardial infarction, 35 patients with significant stenoses or a single stenosis greater than 70 % upon coronary angiography, four patients with positive dipyridamole myocardial thallium 201 scintigraphy and one patient who presented with a positive maximal exercise test. The second group consisted of 67 patients without coronary heart

disease (CHD$^-$), and was composed of 16 patients with no significant stenosis upon coronary angiography, 45 patients whose dipyridamole myocardial thallium scintigraphy test was negative and six patients who had a negative maximal exercise test. Exclusion criteria included renal insufficiency (serum creatinine $\geq$ 130 μmol/l), drug or alcohol abuse, an inflammatory state (sedimentation rate $\geq$ 20 mm/h), a recent myocardial infarction (< 3 months), hepatic disease or neoplasia.

Methods

All examinations were performed at 08.00 hours after an overnight fast. The following clinical data were recorded by the same physician: height, weight, and blood pressure measured after 10 min at rest on the right arm using a mercury sphygmomanometer. Systolic and diastolic blood pressure were recorded to the nearest 2 mm Hg at the first and fifth Korotkoff sounds. Body mass index was calculated as weight/height^2 (kg/m^2). Smoking habits were recorded. Smokers were defined as subjects who regularly smoked more than one cigarette per day, whereas ex-smokers were subjects who had regularly smoked more than one cigarette per day but who had stopped for at least 3 months before the examination; all other subjects were classified as non-smokers. A family history of coronary heart disease was defined as the clinical onset of coronary heart disease in any first degree relative parent or sibling before the age of 60 years. Arterial hypertension was diagnosed according to World Health Organisation criteria ($\geq$ 160/95 mm Hg). Microalbuminuria was defined as an urinary albumin excretion rate between 30 and 300 mg/24 h and macroalbuminuria by an urinary albumin excretion rate of more than 300 mg/24 h for at least two consecutive 24 h urine samples; the expressed results were the mean of two determinations. Urinary albumin concentration was determined by an immunonephelometric method (Behring, Marburg, Germany). Venous blood samples were drawn after an overnight fast. HbA$_{1c}$ was determined by HPLC (DIAMAT Analyser; Bio-Rad, Calif., USA); the normal range of HbA$_{1c}$ in our laboratory is 4.0–6.0 %. Serum cholesterol was measured using CHOP-PAP reagents and triglycerides by an enzymatic method with reagents from Boehringer-Mannheim GmbH (Mannheim, Germany). Serum HDL-cholesterol was estimated enzymatically (Boehringer-Mannheim GmbH), apoB being initially precipitated with phosphotungstate/MgCl$_2$. The quantitation of apoA1 and apoB in whole plasma was performed using commercial antibodies (Behring). Plasma creatinine concentration was assayed by the kinetic method of Jaffe. Lp(a) concentrations were measured in plasma samples stored at –30°C for up to 2 months, using an ELISA method [21]. This assay was developed with two polyclonal antibodies, both prepared in sheep. An anti-human apo(a) antibody [22] was used as the "capture" antibody and a peroxidase-conjugated, anti-human apoB100 antibody as the detecting antibody. Intra- and inter-coefficients of variation for this ELISA assay were 1 % and 2.6 %, respectively. Apo(a) phenotyping was determined according to Kamboh et al. [23] with minor modifications. In particular, apo(a) bands were revealed by use of a chemiluminescent substrate of alkaline phosphatase (AMPPD) [24]. This sensitive method allowed the detection of isoforms present at low concentration and thus the number of null alleles was decreased to 3.6 %. To determine whether CHD$^+$ patients displayed a frequency distribution of apo(a) isoform size which resembled that in CHD$^-$ patients, plasma samples from each individual were subjected to immunoblot analysis. Compared to acrylamide gels, the agarose-gel system can detect a larger number of apo(a) isoforms [23]. It was necessary therefore to develop a nomenclature distinct from that of Utermann [6], as the latter discriminates be-

Table 1. Clinical features in NIDDM patients with (CHD$^+$ group) or without (CHD$^-$ group) coronary heart disease

	CHD$^+$ group $n = 71$	CHD$^-$ group $n = 67$	p-value
Sex (male/female)	59/12	48/19	NS[a]
Age (years)	60.3 ± 8.5	57.9 ± 7.7	NS[b]
Body mass index (kg/m^2)	27.7 ± 3.8	28.5 ± 5.0	NS[b]
Diabetes duration (years)	13.1 ± 8.1	11.5 ± 7.8	NS[b]
Treatment of diabetes mellitus (insulin/oral treatment/diet) (n)	30/29/12	16/42/9	0.031[a]
Hypertension (n)	40	27	NS[a]
Family history of coronary heart disease (n)	15	13	NS[a]
Smokers (nil/ex/active) (n)	23/38/10	26/24/17	NS[a]
Systolic blood pressure (mmHg)	150.3 ± 23.9	150.8 ± 17.5	NS[b]
Diastolic blood pressure (mmHg)	88.2 ± 11.7	92.7 ± 12.6	< 0.03[b]
Anti-hypertensive treatment (n)	67	40	0.0001[a]

Values are mean ± SD. [a] chi-square test, [b] analysis of variance

Table 2. Biological data and plasma lipid levels in NIDDM patients with (CHD$^+$ group) or without (CHD$^-$ group) coronary heart disease

	CHD$^+$ group $n = 71$	CHD$^-$ group $n = 67$	p-value
Glycated haemoglobin (%)	8.4 ± 1.9	7.6 ± 1.6	< 0.01[a]
Creatinine (μmol/l)	95.9 ± 15.0	87.0 ± 12.7	< 0.01[a]
Micro- or macro-albuminuria (n)	32	11	0.0006[b]
Urinary albumin excretion rate[c] (mg/24 h)	30 (5-4620)	9 (5-700)	< 0.01[a]
Plasma lipids (mmol/l):			
Total-cholesterol	6.0 ± 1.0	5.5 ± 1.2	< 0.02[a]
HDL-cholesterol	1.21 ± 0.38	1.17 ± 0.30	NS[a]
Triglycerides	2.1 ± 1.5	1.9 ± 1.2	NS[a]
ApoA1 (g/l)	1.52 ± 0.3	1.50 ± 0.2	NS[a]
ApoB (g/l)	1.41 ± 0.3	1.25 ± 0.3	< 0.01[a]

[a] Analysis of variance with log$_{10}$ transformation for triglycerides and albuminuria, [b] chi-square test, [c] median (range)

tween only six different isoforms. We attributed an arbitrary molecular mass to the four isoforms present in our standard (F, S1, S2 and S3). Since apo B100 (550 kDa) migrated to a position exactly intermediate between that of the F and S1 isoforms, we used values of 500, 600, 700 and 800 kDa for F, S1, S2 and S3, re-

spectively. The apparent molecular mass of the various isoforms detected in patients, plasma was then calculated according to their migration with respect to the standard. For the purpose of this study, we decided to combine apo(a) phenotypes into two groups as proposed by Sandholzer et al. [11]. The first group comprised all subjects who displayed a phenotype with at least one small isoform, i.e. ≤ 700 kDa, a size which corresponds approximately to the S2 isoform in Utermann's classification; such isoforms are generally associated with high Lp(a) levels in a normal population [6]. The second group included all subjects who exhibited one or two apo(a) isoforms larger than 700 kDa, together with those displaying the null phenotype.

Statistical analysis

Data are expressed as mean ± SD, unless stated otherwise. A p value of 0.05 or less was considered as significant. Serum triglycerides, Lp(a) values, apo(a) phenotypes and urinary albumin excretion rate were logarithmically transformed before inclusion in all statistical tests. One way analysis of variance was conducted for continuous variables. Qualitative traits were analysed by contingency table chi-squared. For correlation analysis, Pearson's correlation coefficient was calculated. The potential contribution of apo(a) phenotype to the variance in Lp(a) levels in both CHD$^+$ patients and CHD$^-$ patients was estimated by the R^2 parameter obtained from analysis of variance. As a measure of the possible association between clinical and biological data and coronary heart disease, the odds ratio and 95 % confidence intervals were calculated. Simultaneous adjustments for qualitative and quantitative variables were carried out, using a logistic regression model, in which only variables showing a significant association with coronary heart disease in univariate analysis ($p \leq 0.05$) were introduced. For all calculations, the BMDP Statistical Software (University of California Press, Berkeley, Calif., USA) was used.

Results

The clinical characteristics of NIDDM patients with or without coronary heart disease are shown in Table 1. The CHD$^+$ group included significantly more insulin-treated patients than the CHD$^-$ group. NIDDM patients with coronary heart disease displayed significantly lower diastolic blood pressure than the CHD$^-$ group, although systolic blood pressure did not differ between the groups. The higher frequency of anti-hypertensive treatment in CHD$^+$ patients, introduced principally for secondary cardiovascular prevention, may explain this unexpected result. Indeed, beta-blockers (49 %) and calcium antagonists (67 %) were mainly used in the CHD$^+$ group.

The biological and lipid profiles of our patient groups are shown in Table 2. Glycated haemoglobin, serum creatinine, total cholesterol and apoB were significantly higher in the CHD$^+$ group. In addition, the prevalence of micro- or macroalbuminuria was higher in the CHD$^+$ group (45.1 % vs 16.4 %; $p = 0.0006$).

The overall frequencies of elevated Lp(a) levels did not differ significantly between the sub-groups of patients and controls. Therefore the subsequent analyses were performed on all study subjects. Plasma Lp(a) concentrations and the frequencies of apo(a) phenotypes in

Table 3. Lp(a) concentrations and frequencies of apo(a) phenotypes in NIDDM patients with (CHD$^+$ group) or without (CHD$^-$ group) coronary heart disease

	CHD$^+$ group n = 71		CHD$^-$ group n = 67		p-value
Lp(a)[a] (g/l)	0.29 ± 0.41 (0.11) (0.20–0.39)		0.18 ± 0.28 (0.10) (0.11–0.25)		0.19[b]
Lp(a) ≥ 0.3 g/l (n) (%)	24 (33.8)		9 (13.4)		0.005[c]
Phenotype class (kDa)	%	n	%	n	0.043[c]
≤ 500	0	0	1.49	1	–
≤ 600	1.22	1	2.90	2	–
≤ 700	28.05	20	11.59	8	–
≤ 800	21.95	16	31.88	21	–
≤ 900	28.05	18	42.03	28	–
≤ 1000	15.85	12	8.70	6	–
Null type	5.63	4	1.49	1	–

[a] Mean ± SD (median) (95% confidence interval) [b] analysis of variance after $\log_{10}$ transformation, [c] chi-square test
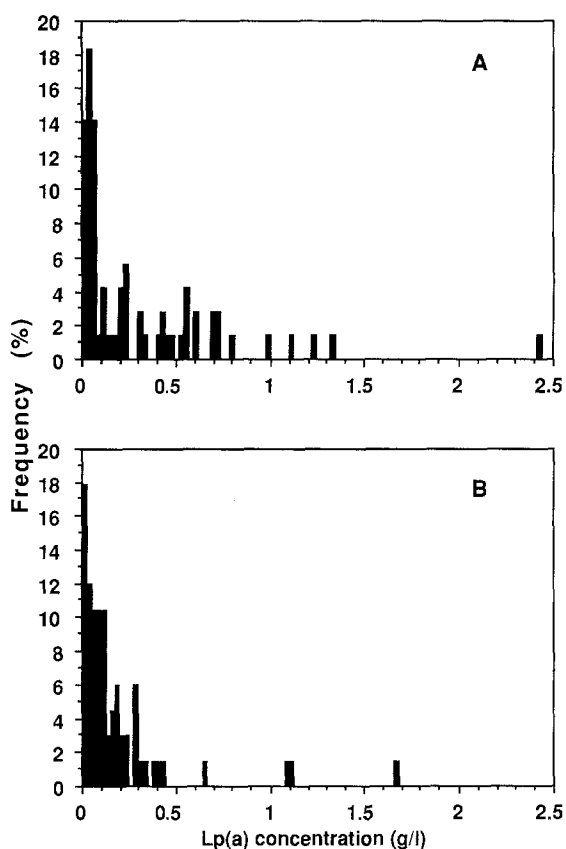
Fig. 1 A, B. Histograms showing the distribution of Lp(a) concentrations in NIDDM patients with (**A**) and without (**B**) coronary heart disease

CHD$^+$ and CHD$^-$ patients are shown in Table 3. There was a positive trend towards higher Lp(a) concentrations in CHD$^+$ patients compared to CHD$^-$ patients; indeed, mean Lp(a) values were 50% higher in patients who have coronary heart disease. Patients with elevated

Lp(a) concentrations (defined as those > 0.3 g/l) were significantly more frequent in CHD$^+$ patients than in CHD$^-$ patients (p = 0.005, Fig. 1).

Hypolipidaemic agents had been prescribed in 23 patients (32.4%) in the CHD$^+$ group and in 10 patients (14.9%) in the CHD$^-$ group (p = 0.016). In the CHD$^+$ group, fibrates and HMGCoA-reductase inhibitors were being taken by 18 and 5 patients, respectively. In the CHD$^-$ group, fibrates and HMGCoA-reductase inhibitors were being taken by 6 and 4 patients, respectively. These two groups of pharmacological agents have not definitively been shown to modify Lp(a) levels but may have significantly influenced other plasma lipid parameters. Lp(a) concentration was not correlated with other clinical and biological parameters in our patient groups when considered either together or separately, with the exception of apoB in CHD$^-$ patients (r = 0.29, p = 0.03).

The overall size range of apo(a) isoforms (480 to 980 kDa) was similar in both patient groups studied. Double-band phenotypes were present in 45 patients (32.6%), and single-band phenotypes in 88 patients (63.8%); the null type was observed in 5 patients (3.6%). The frequency of apo(a) isoforms whose size was below 700 kDa was not significantly greater in CHD$^+$ patients as compared to CHD$^-$ patients (p = 0.067), but a trend toward a predominance of isoforms of low molecular mass was observed in the CHD$^+$ group (29.6%) as compared to the CHD$^-$ group (16.4%). The overall distribution of apo(a) phenotypes was however significantly displaced towards low molecular mass isoforms in the CHD$^+$ group (p = 0.043; Table 3). In both populations, apo(a) isoforms were significantly associated with Lp(a) levels (r = –0.58, p = 0.0001 for CHD$^+$ and r = –0.34, p = 0.0049 for CHD$^-$), though mean Lp(a) levels and the frequency distribution of apo(a) phenotypes were distinct between the two groups. Thus, the inverse relation observed by Utermann et al. [6] between the size of the apo(a) isoform and Lp(a) concentration in plasma also exists in our population of diabetic patients, either with or without coronary heart disease. We subsequently estimated the contribution of apo(a) phenotypes to the inter-individual variation in Lp(a) concentrations by R^2 from analysis of variance. The fraction of the variance in Lp(a) levels which was explained by apo(a) isoforms was two-fold larger in the CHD$^+$ group as compared to the CHD$^-$ group (0.28 and 0.15, respectively).

Univariate analyses, with cardiovascular status as the categorical dependent variable, were performed to evaluate a possible association of clinical and biological parameters with the risk of coronary heart disease (Table 4). Elevated Lp(a) values were significantly associated with coronary heart disease, giving an odds ratio of 3.1. Although not statistically significant (p = 0.067), a trend toward an association between apo(a) phenotypes of low molecular mass and the risk of coronary heart disease was observed. A significant

Table 4. Crude odds ratio of coronary heart disease in relation to cardiovascular risk factors

	Odds ratio	95 % confidence interval	p-value
Lp(a) (≥ 0.3 g/l)	3.10	1.31– 7.34	0.010
Apo(a) phenotypes ≤ 700 kDa	2.14	0.95– 4.82	0.067
Age (years)	1.04	0.99– 1.08	0.116
Sex (male/female)	2.01	0.87– 4.67	0.104
Smokers (yes/no)	1.42	0.70– 2.87	0.330
Micro- and macro-albuminuria (yes/no)	4.54	2.02–10.19	< 0.001
HbA$_{1c}$ (%)	1.34	1.09– 1.66	0.007
Creatinine (μmmol/l)	1.05	1.02– 1.08	< 0.001
Total cholesterol (mmol/l)	1.68	1.19– 2.39	0.004
ApoB (g/l)	6.71	1.93–23.37	0.003

Table 5. Logistic regression analysis of cardiovascular risk factors associated with coronary heart disease in univariate analysis

	Odds ratio	95 % confidence interval	p-value
Lp(a) (≥ 0.3 g/l)	3.48	1.19–10.22	0.0233
Micro- and macro-albuminuria	3.37	1.18– 9.63	0.0230
Creatinine	1.05	1.01– 1.09	0.0115
ApoB	6.54	0.62–68.76	0.1178
Insulin treatment	0.68	0.40– 1.15	0.1524
Glycated haemoglobin	1.17	0.87– 1.58	0.3085
Total cholesterol	0.93	0.48– 1.79	0.8279

association was observed between creatinine, micro- or macroalbuminuria, HbA$_{1c}$ and coronary heart disease. Total cholesterol and apoB were also significantly associated with coronary heart disease. The other lipid and apolipoprotein parameters (triglycerides, HDL-cholesterol and apoA1) did not show any significant association with coronary heart disease.

According to the univariate analyses, high Lp(a) levels were found to be a risk factor for coronary heart disease in this NIDDM population. A multiple logistic regression analysis was performed (Table 5), introducing the cardiovascular risk factors for coronary heart disease that were identified in previous analyses (p value < 5 %). In our NIDDM population, high Lp(a) levels appeared to be an independent cardiovascular risk factor (odds ratio 3.48, p = 0.0233). High Lp(a) levels contribute to increased risk of coronary heart disease independently of micro- or macroalbuminuria and creatinine; the latter parameters which were also independently associated with coronary heart disease in this study. It must be emphasized that the association between high Lp(a) levels and coronary heart disease was significant in NIDDM patients without micro- and macroalbuminuria (odds ratio 3.97, p < 0.01) but not in the sub-group with diabetic nephropathy (odds ratio 1.76, NS) (data not shown).

Discussion

In this case-control study, we have evaluated the relation between Lp(a) levels, apo(a) phenotypes and coronary heart disease in a Caucasian population of NIDDM patients. We found a significant and independent association between elevated Lp(a) levels and the presence of coronary heart disease in these patients when a plasma Lp(a) level of 0.3 g/l was used as the cut-off; this level was defined as the threshold for high cardiovascular risk [25] and correspond to the 75th percentile in our laboratory (data not shown). This finding was not a consequence of diabetic nephropathy as significant differences were observed when patients without nephropathy were examined separately. Conversely, differences were not significant when patients with micro- or macroalbuminuria were examined, although fewer patients were included in this group. We also observed a trend toward high Lp(a) concentrations in CHD[+] patients, in agreement with other studies involving non-diabetic populations [9, 25, 26]. The lack of significance which we observed may be due to the relatively small size of the study population and could also be due to its cross-sectional design. This latter point may be important, because elevated Lp(a) concentrations could lead to premature cardiovascular death.

The use of lipid-lowering therapy was not considered as an exclusion criterion. The impact of the different lipid-lowering drug therapies may thus have contributed to the negative results concerning the association between triglycerides, HDL-cholesterol and coronary heart disease in this study. In addition, the relatively small size of the population may also be a factor. With the exception of nicotinic acid [27], no hypolipidaemic agent has as yet been proven to consistently modify circulating Lp(a) levels. Similarly, anti-hypertensive treatment, such as beta-blockers which are known to modify plasma lipid levels, was more frequent in the CHD[+] group. To our knowledge, a relationship between anti-hypertensive treatment and Lp(a) concentrations has not been firmly established; nonetheless, we cannot exclude a possible influence of anti-hypertensive treatment on Lp(a) levels. In particular, angiotensin-converting enzyme may improve lipid abnormalities in NIDDM hypertensive patients [28]. In our study, the percentage of patients treated with angiotensin-converting enzyme inhibitor was significantly higher in the CHD[-] group than in CHD[+] group (68 % vs 33 %, p < 0.01, data no shown).

Two studies in NIDDM patients did not detect an association between Lp(a) levels and coronary heart disease [14, 15]. Survival bias might however explain these negative results, as well as misclassification of NIDDM patients with or without coronary heart disease. Clinical examination and/or resting electrocardiography provide low sensitivity and specificity in such patients. Some of the presumed diabetic subjects without coronary heart disease may have had clinically undetected

coronary artery disease which frequently occurs in diabetic patients [18]. In the present study, we therefore used both non-invasive tests and coronary angiography to extensively evaluate coronary artery disease.

We found that the global distribution of apo(a) phenotypes differed significantly between the CHD⁺ and CHD⁻ groups. The frequency of apo(a) phenotypes 700 kDa or less was two-fold higher in CHD⁺ patients compared to that in CHD⁻ patients, but did not reach statistical significance. When the distribution of apo(a) phenotypes in NIDDM patients was compared with that in non-diabetic control subjects by Csaszar et al. [13], no significant difference was shown, suggesting that apo(a) phenotype and incidence of diabetes are independent. Nonetheless, our present results suggest that Lp(a) concentrations in NIDDM patients are genetically controlled as in non-diabetic Caucasians, and consequently, could potentially contribute to coronary heart disease. The contribution of apo(a) phenotypes to Lp(a) levels was greater in CHD⁺ patients than in the CHD⁻ group. A similar observation was previously reported in normal subjects by Sandholzer et al. [11]. In addition, size of apo(a) isoform may also be a contributing factor to its cardiovascular effects [11, 29]. Other genetic factors might also influence Lp(a) concentrations. Indeed, a recent study [30] has shown that the apo(a) gene is more polymorphic in sequence than previously thought based on the number of kringle 4 repeats. However, a variety of non-genetic factors has also been shown to affect Lp(a) concentrations; some of which are associated with increased coronary heart disease prevalence such as menopause [31] or end-stage renal disease [32]. We previously demonstrated [21] that Lp(a) levels are elevated in end-stage renal disease, but we did not detect any association with diabetes in this population. Consequently, diabetic patients who had renal insufficiency were excluded from the present study. Given this restriction, no correlation was observed between albuminuria and Lp(a) concentrations. Data on the possible association between these two variables are inconclusive in NIDDM, as both positive [33] and negative findings have been reported [34, 35].

Other classic cardiovascular risk factors were also associated with coronary heart disease in this study, and these include micro- or macroalbuminuria or both, which are well established as independent cardiovascular risk factors in NIDDM [36]. Plasma total cholesterol and apoB levels were also significantly associated with coronary heart disease in our patients, despite the more frequent use of hypolipidaemic treatment in the CHD⁺ group.

In conclusion, our study has demonstrated a positive and independent association between elevated Lp(a) levels and coronary heart disease in NIDDM Caucasian patients. Furthermore, the observed difference in the distribution of apo(a) phenotypes between CHD⁻ and CHD⁺ patients is consistent with suggestions that the

apo(a) gene locus influences the risk for coronary heart disease in diabetic patients through its effect on Lp(a) levels [11]. Diabetic nephropathy was not a contributory factor to the increased Lp(a) levels in NIDDM patients. Large, long-term, prospective studies are now required to eliminate survival bias and to assess whether high Lp(a) levels and apo(a) phenotypes are associated with the incidence of coronary heart disease and other vascular complications in NIDDM patients.

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