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Plasma apolipoprotein (a) is increased in Type 2 (non-insulin-dependent) diabetic patients with microalbuminuria

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Summary. Patients with Type 2 (non-insulin-dependent) diabetes mellitus complicated by microalbuminuria or albuminuria, have an increased risk of developing macrovascular disease and of early mortality. Because lipoprotein abnormalities have been associated with diabetic nephropathy, this study tested the hypothesis that levels of apolipoprotein (a) are elevated in patients with Type 2 diabetes and increased levels of urinary albumin loss. Levels of apolipoprotein (a) in diabetic patients with microalbuminuria ($n = 26$, geometric mean 195 U/l, 95% confidence interval 117–324) and albuminuria ($n = 19$, 281 U/l, 165–479) were higher than in non-diabetic control subjects ($n = 140$, 107 U/l, 85–134, $p < 0.05$), and in the albuminuric group than diabetic patients without urinary albumin loss ($n = 58$, 114 U/l, 76–169, $p < 0.05$). Patients with microalbuminuria and albuminuria had levels comparable with patients undergoing elective coronary artery graft surgery ($n = 40$, 193 U/l, 126–298). Apolipoprotein (a) levels were higher in diabetic patients with macrovascular disease than in those without ($n = 49$, 209 U/l, 143–306 vs $n = 54$, 116 U/l, 78–173, $p < 0.05$). These preliminary results suggest that raised apolipoprotein (a) levels of Type 2 diabetic patients with microalbuminuria and albuminuria may contribute to their propensity to macrovascular disease and early mortality.

Key words: Apolipoprotein (a), microalbuminuria, Type 2 (non-insulin-dependent) diabetes mellitus, macrovascular disease.

There is evidence that microalbuminuria in Type 2 (non-insulin-dependent) diabetic patients predicts the development of proteinuria and early mortality [1, 2]. The excess morbidity and mortality of Type 2 diabetic patients is predominantly due to macrovascular disease, with renal failure accounting for only 3–5% of total mortality [3, 4]. Potential contributory factors to the excess macrovascular disease include atherogenic changes in the lipid profile, increased blood pressure, hyperglycaemia, hyperinsulinaemia, obesity and alteration of haemostatic factors [3, 5].

Lipoprotein (a) or Lp (a) is an independent risk factor for macrovascular disease, the levels of which are under strong genetic influence. Structurally it is a hybrid of low density lipoprotein (LDL) and a specific plasminogen-like glycoprotein apolipoprotein (a) or apo (a), which is attached to the apo B100 of LDL [6]. Recent reports have indicated that apo (a) levels are elevated in Type 1 (insulin-dependent) diabetic patients with microalbuminuria and albuminuria [7–9]. In this study we have investigated whether apo (a) levels are also raised in Type 2 diabetic patients with increased urinary albumin loss, which may contribute to the heightened risk of macrovascular disease in these patients.

Patients and methods

Our study comprised 103 patients with Type 2 diabetes [10] attending the St Vincent's Hospital Diabetes Clinic. All subjects gave informed consent and the study was approved by the Human Research Ethics Committee at St Vincent's Hospital. Cigarette smokers were defined as current smokers or those having smoked more than one cigarette per day within the previous 5 years. Macrovascular disease included ischaemic heart, peripheral vascular and cerebrovascular disease. Positive manifestations were a resting 12-lead ECG with evidence of infarction, or a history of angina, myocardial infarction, transient ischaemic attack, stroke, intermittent claudication, rest pain, limb amputation or arterial surgery. All patients who had absent pedal pulses on examination also had other evidence of macrovascular disease. Patients with intercurrent conditions or taking medications that might influence apo (a) levels [11–14] were excluded from sampling.

Urine collections were analysed for albumin (rate nephelometry, coefficient of variation, CV < 5%), total protein and creatinine. The mean concentration from two overnight 12-h urine collections performed on consecutive days, not after heavy exercise, was taken. The Type 2 diabetic population was subdivided according to the level of urinary albumin loss: less than or equal to 20 μg/min, no microalbuminuria, between 21 and 200 μg/min, microalbuminuria, and greater than 200 μg/min, albuminuria. After a 10 to 12 h overnight fast 10 ml of blood was taken by venepuncture into an EDTA tube for lipid anal-

Table 1. Characteristics of the Type 2 (non-insulin-dependent) diabetic population subdivided according to urinary albumin loss

	Non-microalbuminuric patients (≤ 20 μg/min)	Microalbuminuric patients (21–200 μg/min)	Albuminuric patients (> 200 μg/min)
Number (male/female)	27/31	15/11	12/7
Age (years)	59 (55–62)	59 (55–64)	61 (56–65)
Diabetes duration (years)	10 (8–12)	10 (8–14)	14 (9–19)
BMI (kg/m^2)	28.1 (27.0–29.3)	28.5 (26.9–30.2)	31.0 (29.0–33.0)[a]
Diet/oral hypoglycaemic agents/insulin (%)	5/60/35	8/42/50	5/26/69
HbA_{1c} (%)	8.3 (7.7–8.9)	7.6 (6.8–8.4)	7.5 (6.7–8.3)
Macrovascular disease (%)	40	62	53
Hypertension (%)	48	65	84[a]
Smokers (%)	24	35	42
Total cholesterol (mmol/l)	6.5 (6.1–6.9)	6.6 (5.9–7.3)	6.8 (6.0–7.7)
Triglyceride (mmol/l)	2.0 (1.6–2.4)	2.1 (1.7–2.7)	2.2 (1.7–2.8)
HDL cholesterol (mmol/l)	1.1 (1.0–1.2)	1.0 (0.9–1.1)	1.1 (1.0–1.3)

[a] $p < 0.05$ vs non-microalbuminuric patients
Values are arithmetic or geometric mean (95 % confidence interval)

Table 2. Parameters of renal function, plasma protein and albumin in the Type 2 (non-insulin-dependent) diabetic population subdivided according to urinary albumin loss

Parameter (Reference range)	Non-microalbuminuric patients (≤ 20 μg/min)	Microalbuminuric patients (21–200 μg/min)	Albuminuric patients (> 200 μg/min)
Plasma urea (3.1–8.3 mmol/l)	5.9 (5.3–6.4)	6.7 (5.7–7.9)	7.8 (6.4–9.5)[a]
Plasma creatinine (0.07–0.11 mmol/l)	0.09 (0.08–0.09)	0.09 (0.09–0.10)	0.11 (0.10–0.13)[b]
Creatinine clearance (1.5–2.5 ml/sec)	1.4 (1.2–1.6)	1.3 (1.0–1.6)	1.1 (0.8–1.5)
Urinary albumin (≤ 20 μg/min)	≤ 20	52 (38–87)[b]	705 (433–1221)[bc]
Urinary protein (< 0.10 g/day)	0.04 (0.04–0.05)	0.09 (0.07–0.12)[b]	0.59 (0.45–0.80)[bd]
Plasma protein (60–80 g/l)	74 (73–76)	76 (74–78)	74 (71–77)
Plasma albumin (35–50 g/l)	43 (42–44)	45 (43–46)	43 (41–44)

[a] $p < 0.05$; [b] $p < 0.001$ vs non-microalbuminuric patients; [c] $p < 0.001$; [d] $p < 0.01$ vs microalbuminuric patients
Values are arithmetic or geometric mean or for urinary albumin, median (95 % confidence interval)

yses and 10 ml into a lithium heparin tube for other measurements. Parameters assayed were HbA_{1c}, total cholesterol, triglyceride, HDL cholesterol, total protein, albumin and creatinine. Apo (a) was measured by immunoradiometric assay (Pharmacia, Uppsala, Sweden) as described previously [7]. LDL cholesterol was calculated using a modification of the Friedewald equation that corrects for the cholesterol content of Lp (a). The formula used [12] was:

LDL (mmol/l) = total cholesterol – HDL cholesterol-triglyceride/2.2 – Lp (a) (mg/l) × 0.3/386.7, assuming that 1 U/l apo (a) = 1 mg/l Lp (a).

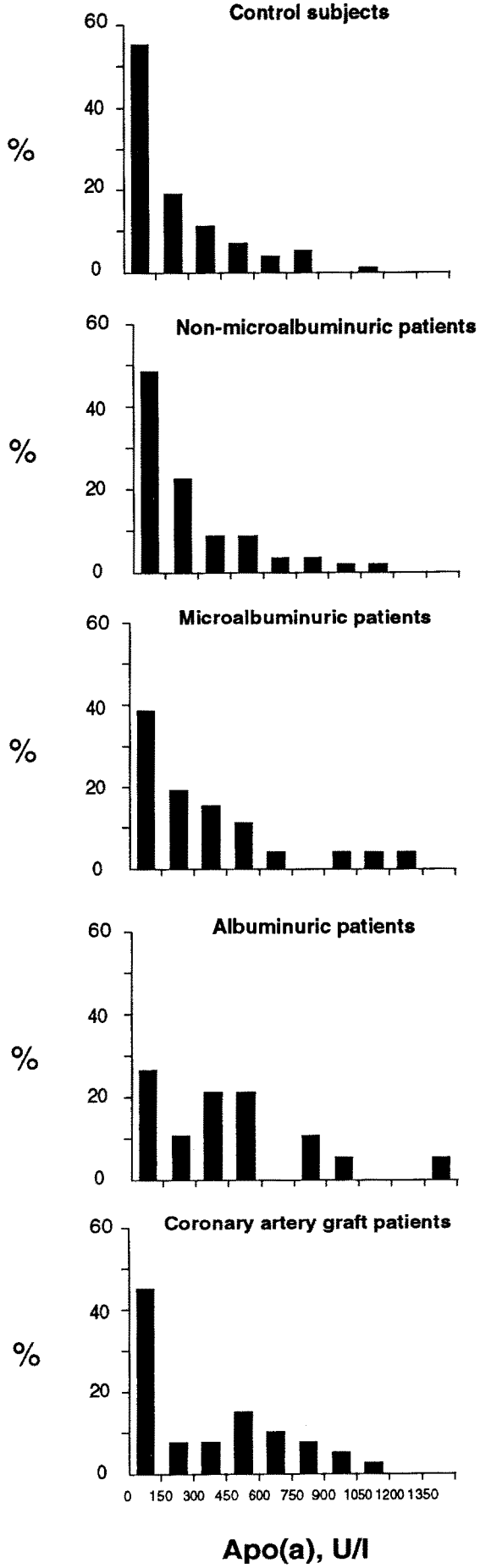
Apo (a) levels were also determined in a control population of 140 non-diabetic apparently healthy hospital staff members (62 men and 78 women), of whom 44 had fasted for 10–12 h. In addition apo (a) was measured in fasting blood samples from 40 consecutive non-diabetic men about to undergo elective coronary artery graft surgery for angiographically diagnosed coronary artery disease. Apo (a) levels in these different groups can be compared as age, gender, body mass index and prandial status do not influence apo (a) levels [12–15]. The predominant ethnic background of all study subjects was Caucasian [16].

Statistical analysis

Statistical analyses were performed using the MINITAB (Minitab Inc., State College, Pa., USA) STATVIEW and SUPERANOVA (Abacus Concepts, Berkeley, Calif., USA) statistical packages. Skewed distributions were first normalized using square root or logarithmic transformations. Analysis of variance was used to compare continuous variables and the chi square test to compare discontinuous variables between groups. Correlations between normalized variables were tested by the Pearson correlation test.

Results

Characteristics of the Type 2 diabetic population divided according to urinary albumin loss are shown in Tables 1 and 2. As shown in Figures 1 and 2, apo (a) levels were increased in the Type 2 diabetic patients in the microalbuminuria group (geometric mean 195 U/l, 95 % confidence interval 117–324) and albuminuria group (281 U/l, 165–479) relative to control subjects (107 U/l, 85–134) and in the albuminuric group relative to diabetic patients without microalbuminuria (114 U/l, 76–169). Apo (a) levels in the diabetic microalbuminuria and albuminuria groups were comparable with those of the non-diabetic patients undergoing coronary artery graft surgery (193 U/l, 126–298). Apo (a) levels were higher in Type 2 diabetic patients with macrovascular disease ($n = 49$, 209 U/l, 143–306) than in those without ($n = 54$, 116 U/l, 78–173,



$p < 0.05$). This finding was independent of urinary albumin loss grouping as determined by two factor analysis of variance.

In the Type 2 diabetic patients log apo (a) correlated with age ($r = 0.234$, $p < 0.05$) and with log plasma creatinine ($r = 0.327$, $p < 0.001$). However, log plasma creatinine correlated with age ($r = 0.234$, $p < 0.05$) and when these two variables were used in multiple regression analysis, age was no longer a significant predictor. There was a negative relationship between log apo (a) and the square root of creatinine clearance ($r = -0.188$), which was not statistically significant ($0.10 > p > 0.05$). There was no correlation between log apo (a) and the amount of urinary protein or albumin loss.

Analysis of variance and covariance were also used to explore the determinants of log apo (a) values. Single significant factors included urinary albumin grouping (Fisher distribution value [F] = 3.57, $p < 0.05$), and the covariate log plasma creatinine (F = 11.64, $p < 0.001$). Plasma protein was not significant as a single covariate (F = 2.18, $p > 0.05$), although it was significant when included in the model with log plasma creatinine (log plasma creatinine F = 13.76, $p < 0.001$, plasma protein F = 4.71, $p < 0.05$).

Discussion

We have demonstrated increased apo (a) levels in a limited number of Type 2 diabetic patients with microalbuminuria and albuminuria relative to non-diabetic control subjects, and in the albuminuric group relative to diabetic patients without urinary albumin loss. Elevation of Lp (a) with hyperglycaemia has been reported for Type 1 [16, 17] but not Type 2 diabetes [18]. We found no relationship between glycaemic control and level of apo (a) in this study. Consistent with the proposed role for Lp (a) as an atherogenic particle, apo (a) levels were higher in Type 2 diabetic patients with macrovascular disease than in those without, independent of urinary albumin loss. Similar findings have been reported previously for predominantly non-diabetic subjects [19], although a recently reported prospective study did not show any association between coronary heart disease mortality and Lp (a) in a small number of patients with Type 2 diabetes [20].

Elevated apo (a) or Lp (a) levels have been shown to be associated with coronary artery disease, cerebral infarction, cervical atheroma, aortic aneurysms and peripheral vascular disease [21–25]. One mechanism by which Lp (a) could accelerate atherosclerosis is by penetration into the arterial wall [21, 25, 26]. Another possible atherogenic effect is attenuation of clot lysis due to competi-

Fig. 1. Frequency histograms of apolipoprotein (a) levels in the control subjects ($n = 140$), patients with Type 2 (non-insulin-dependent) diabetes divided according to urinary albumin loss (non-microalbuminuric, $n = 58$; microalbuminuric, $n = 26$; albuminuric, $n = 19$) and coronary artery graft patients ($n = 40$)

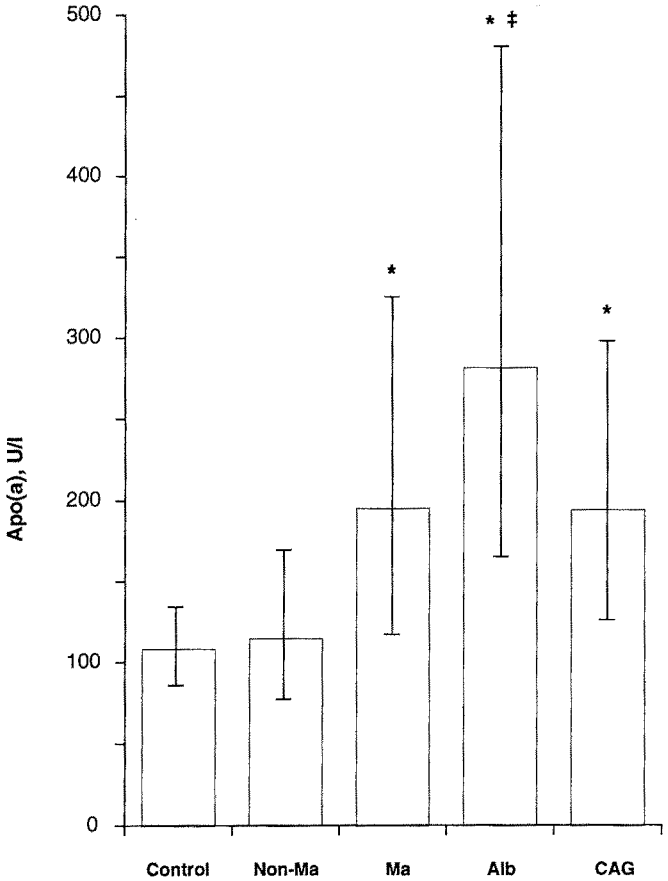


Fig. 2. Geometric mean and 95 % confidence intervals of the control subjects ($n = 140$), patients with Type 2 (non-insulin-dependent) diabetes divided according to urinary albumin loss (non-microalbuminuric, Non-Ma, $n = 58$; microalbuminuric, Ma, $n = 26$; albuminuric, Alb, $n = 19$) and coronary artery graft patients (CAG, $n = 40$). Apolipoprotein (a) [apo (a)] levels were compared by analysis of variance. * $p < 0.05$ vs control subjects; ‡ $p < 0.05$ vs Non-Ma patients

tive binding of apo (a) to plasminogen receptors on the vascular endothelium, reducing activation of plasminogen by tissue plasminogen activator [21, 27]. Thus, elevated apo (a) may be an important factor in the observed link between microalbuminuria, macrovascular disease and early mortality in Type 2 diabetic patients [1]. Prospective studies will be necessary to define the importance of this association.

Diabetic nephropathy in Type 2 diabetes has not been studied extensively and it is not yet clear whether the incidence and rate of progression is the same in Type 2 as in Type 1 diabetes. It has been suggested that microalbuminuria in the older non-diabetic population may be a manifestation of widespread atherosclerosis [28] and it remains possible that the association in this study between elevated apo (a) and microalbuminuria in Type 2 diabetes is due to the common link with macrovascular disease. However, development of microalbuminuria in Type 1 diabetes also predicts an increased risk of cardiovascular disease [29] and has been shown to be associated with elevation of apo (a) levels [7–9].

The link between diabetic renal disease and increased apo (a) levels was reinforced in this study by the finding that log plasma creatinine was a significant predictor of log apo (a) levels. Elevated apo (a) levels have been observed previously in patients with chronic renal failure [14, 30] and have been shown to decrease after renal transplantation [30]. In our study, renal function, as evaluated by plasma urea, creatinine and creatinine clearance, was relatively normal and certainly none of the patients had end-stage renal failure. Although the findings of this study indicate a likely association between apo (a) and diabetic nephropathy, we cannot deduce whether raised apo (a) is an effect of altered renal function, the cause of renal damage, or both.

If elevated apo (a) is due to disturbed renal function, potential mechanisms may involve increased urinary protein loss. In the nephrotic syndrome, urinary protein loss or changes in plasma albumin may trigger increased hepatic production of lipoproteins [31, 32], and elevated levels of Lp (a) have been reported in patients with heavy proteinuria, but normal creatinine [33]. On the other hand, a role for hyperlipidaemia in chronic renal injury has been postulated [34, 35]. Mechanisms for renal damage by Lp (a) could include enhanced atherosclerosis and microthrombi formation which are recognised histological features of diabetic nephropathy and consistent with the pathophysiological role postulated for Lp (a) [21, 25].

Longitudinal studies will help to resolve the issue of whether raised Lp (a) is a consequence or a cause of diabetic renal disease. It will be of particular interest to observe whether apo (a) rises with the onset and progression of microalbuminuria and whether measures that reduce urinary albumin loss also reduce apo (a) levels. Alternatively, extensive family studies with phenotyping could be used to establish whether elevated apo (a) is a genetically determined or acquired characteristic of the patients with diabetic nephropathy. Although many questions about the relationship remain unanswered, our preliminary results suggest that apo (a) levels are elevated in Type 2 diabetic patients with renal damage, even at its very early stage of microalbuminuria. Thus, we have identified a further risk factor that may contribute to the well-recognised high risk of vascular disease and early mortality in Type 2 diabetic patients. There is evidence that Lp (a) level is reduced by treatment with niacin, neomycin, omega-3-fatty acids and stanazolol [11, 12], however, it is not yet known whether such treatment in people with high levels of apo (a) will reduce the incidence of vascular disease.

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References

1. Mogensen CE (1984) Microalbuminuria predicts clinical proteinuria and early mortality in maturity onset diabetes. N Engl J Med 310: 356–360

2. Jarrett RJ, Viberti GC, Argyropoulos A, Hill RD, Mahmud U, Murrells TJ (1984) Microalbuminuria predicts mortality in non-insulin-dependent diabetes. Diabetic Med 1: 17–19
3. Panzram G (1987) Mortality and survival in type 2 (non-insulin dependent) diabetes mellitus. Diabetologia 30: 123–131
4. Mattock MB, Keen H, Viberti GC et al. (1988) Coronary heart disease and urinary albumin excretion rate in type 2 (non-insulin-dependent) diabetic patients. Diabetologia 31: 82–87
5. Pyörälä K, Laakso M, Uusitupa M (1987) Diabetes and atherosclerosis: an epidemiologic view. Diabetes Metab Rev 3: 464–524
6. Utermann G (1989) The mysteries of lipoprotein (a). Science 246: 904–910
7. Jenkins AJ, Steele JS, Janus ED, Best JD (1991) Increased plasma apolipoprotein (a) levels in type 1 diabetic patients with microalbuminuria. Diabetes 40: 787–790
8. Winocour PH, Bhatnagar D, Ishola M, Arrol S, Durrington PN (1991) Lipoprotein (a) and microvascular disease in type 1 (insulin-dependent) diabetes. Diabetic Med 8: 922–927
9. Kapelrud H, Bangstad HJ, Dahl-Jorgensen K, Berg K, Hanssen KF (1991) Serum Lp (a) lipoprotein concentrations in insulin dependent diabetic patients with microalbuminuria. Br Med J 303: 675–678
10. National Diabetes Data Group (1979) Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28: 1029–1057
11. Kostner GM (1990) The physiological role of Lp (a). In: Scanu A (ed) Lipoprotein (a). Academic Press, San Diego, pp 183–204
12. Dahlen GH (1990) Incidence of Lp (a) lipoprotein among populations. In: Scanu A (ed) Lipoprotein (a). Academic Press, San Diego, pp 151–173
13. Maeda S, Abe A, Seishima M, Makino K, Noma A, Kawade M (1989) Transient changes of serum lipoprotein (a) as an acute phase protein. Atherosclerosis 787: 145–150
14. Parra HJ, Mezdour H, Cachera C, Dracon M, Tacquet A, Fruchart JC (1987) Lp (a) lipoprotein in patients with chronic renal failure treated by hemodialysis. Clin Chem 33: 721
15. Albers JJ (1990) The measurement of Lp (a) and its clinical application. In: Scanu A (ed) Lipoprotein (a). Academic Press, San Diego, pp 141–149
16. Levitsky LL; Scanu AM, Gould SH (1991) Lipoprotein (a) levels in black and white children and adolescents with IDDM. Diabetes Care 14: 283–287
17. Bruckert E, Davidoff P, Grimaldi A et al. (1990) Increased serum levels of lipoprotein (a) in diabetes mellitus and their reduction with glycemic control. JAMA 263: 35–36 (Letter)
18. Haffner SM, Tuttle KR, Rainwater DL (1992) Lack of change of lipoprotein (a) concentration with improved glycemic control in subjects with type II diabetes. Metabolism 41: 116–120
19. Rees A, Bishop A, Morgan R (1990) The apo (a) gene: structure/function relationships and the possible link with thrombotic atheromatous disease. Br Med Bull 46: 873–890
20. Haffner SM, Moss SE, Klein BEK, Klein R (1992) Lack of association between lipoprotein (a) concentrations and coronary heart disease mortality in diabetes: the Wisconsin epidemiologic study of diabetic retinopathy. Metabolism 41: 194–197
21. Mbewu AD, Durrington PN (1990) Lipoprotein (a): structure, properties and possible involvement in thrombogenesis and atherogenesis. Atherosclerosis 85: 1–4
22. Rhoards GG, Dahlen G, Berg K, Morton NE, Darrenberg AL (1986) Lp (a) lipoprotein as a risk factor for myocardial infarction. JAMA 256: 2540–2544
23. Murai A, Mijahara T, Fujimoto N, Matsuda M, Kameyama M (1986) Lp (a) lipoprotein as a risk factor for coronary heart disease and cerebral infarction. Atherosclerosis 59: 199–204
24. Koltringer P, Jurgens G (1985) A dominant role of lipoprotein (a) in the investigation and evaluation of parameters indicating the development of cervical atherosclerosis. Atherosclerosis 58: 187–198
25. Loscalzo J (1990) Lipoprotein (a). A unique risk factor for atherothrombotic disease. Arteriosclerosis 10: 672–679
26. Cushing GL, Gaubatz JW, Nava ML et al. (1989) Quantitation and localization of apolipoproteins (a) and B in coronary artery bypass vein grafts resected at reoperation. Arteriosclerosis 9: 593–603
27. Miles LA, Fless GM, Levin EG, Scanu AM, Plow EF (1989) A potential basis for the thrombotic risks associated with lipoprotein (a). Nature (Lond) 339: 301–305
28. Yudkin JS, Forrest RD, Jackson CA (1988) Microalbuminuria as a predictor of vascular diseases in non-diabetic subjects. Lancet II: 530–533
29. Borch-Johnsen K, Kreiner S (1987) Proteinuria: value as predictor of cardiovascular mortality in insulin-dependent diabetes mellitus. Br Med J 294: 1651–1654
30. Black IW, Wilcken DEL (1992) Decreases in apolipoprotein (a) after renal transplantation: implications for lipoprotein (a) metabolism. Clin Chem 38: 353–357
31. Appel GB, Blum CB, Chien S, Kunis CL, Appel AS (1985) The hyperlipidaemia of the nephrotic syndrome. Relation to plasma albumin concentration, oncotic pressure, and viscosity. N Engl J Med 312: 1544–1548
32. Joven J, Villabona C, Vibella E, Masana L, Albert R, Valles M (1990) Abnormalities of lipoprotein metabolism in patients with the nephrotic syndrome. N Engl J Med 323: 579–584
33. Karadi I, Romics L, Palos G et al. (1989) Lp (a) lipoprotein concentration in serum of patients with heavy proteinuria of different origin. Clin Chem 35: 2121–2123
34. Moorhead JF, Chan MK, El-Nahos M, Varghese Z (1982) Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. Lancet II: 1309–1311
35. Kasiske BL, O'Donnell MP, Cowardin W, Keane WF (1990) Lipids and the kidney. Hypertension 15: 443–450

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