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Serum Lp(a) lipoprotein concentrations in insulin dependent diabetic patients with microalbuminuria.

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Abstract

Objective—To compare the serum concentrations of lipoproteins and apolipoproteins in insulin dependent diabetic patients with and without microalbuminuria.

Design—Cross sectional study.

Setting—Paediatric and medical outpatient clinic at a university hospital.

Patients—76 insulin dependent diabetic patients: 41 with microalbuminuria (20 males, 21 females) and 35 controls without microalbuminuria (18 males, 17 females). The two groups were similar with respect to age, duration of disease, and haemoglobin A_{1c} concentrations before the study.

Main outcome measures—Serum concentrations of Lp(a) lipoprotein, total cholesterol, high density lipoprotein cholesterol, very low density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides, and apolipoproteins A-I, A-II, and B.

Results—Median serum Lp(a) lipoprotein concentration was 10.0 mg/100 ml in the microalbuminuric group and 4.9 mg/100 ml in the control group ($p=0.007$). 17 (41%) of the microalbuminuric patients and five (14%) of the control patients had Lp(a) lipoprotein values above the upper quartile of a normal population. Median serum triglycerides concentrations in the microalbuminuric and control groups were 1.15 mmol/l and 0.88 mmol/l respectively ($p=0.03$). Median very low density lipoprotein cholesterol concentration was 0.52 mmol/l in the microalbuminuric group and 0.40 mmol/l in the control group ($p=0.03$). No significant differences in serum concentrations of total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, or apolipoproteins A-I, A-II, and B were found between the groups.

Conclusions—Serum concentrations of Lp(a) lipoprotein are twice as high in insulin dependent diabetic patients with microalbuminuria as in those without microalbuminuria. Increased concentrations of Lp(a) lipoprotein might partly explain the increased morbidity and mortality from cardiovascular disease observed among patients with diabetic nephropathy.

Introduction

Mortality is 40 times higher in insulin dependent diabetic patients with nephropathy than in those without this complication.¹ Much of the excess mortality is caused by cardiovascular disease.² Forty per cent of diabetic patients develop coronary heart disease within six years after manifesting nephropathy.³ Such patients have increased blood pressure, atherogenic lipid and lipoprotein patterns, and changes in the

balance between coagulation and fibrinolysis in favour of coagulation,^{4,6} but these cardiovascular risk factors cannot explain the substantial increase in mortality.⁷ Patients with incipient nephropathy (persistent microalbuminuria) may have the same cardiovascular risk factors, but to a lesser extent.^{4,8} Diabetic patients with incipient nephropathy are at risk of developing proteinuria within 10 years.^{9,10}

Lp(a) lipoprotein contains less lipid and more protein than low density lipoprotein, although both contain apolipoprotein B. Lp(a) lipoprotein has a unique long, highly glycosylated polypeptide chain (Lp(a) apolipoprotein) which carries the Lp(a) antigens. The gene for Lp(a) apolipoprotein has been cloned and extensive homology with plasminogen has been found.¹¹

The concentration of Lp(a) lipoprotein in serum is determined genetically.¹²⁻¹⁶ Since the first report in 1974,¹⁷ several investigators have confirmed that a high Lp(a) lipoprotein concentration is an independent risk factor for cardiovascular disease.¹⁶⁻²⁰ Lp(a) lipoprotein has been detected in atherosclerotic lesions in amounts reportedly reflecting the serum concentration,²¹ suggesting that it may be atherogenic. Because of the homology with plasminogen it has been hypothesised that the Lp(a) lipoprotein may provide a link between atherogenesis and thrombus formation.²²

As diabetic nephropathy is linked with atherosclerotic disease and as the diabetic patients with microalbuminuria are those who develop nephropathy, we investigated whether insulin dependent diabetic patients with microalbuminuria have an increased plasma concentration of Lp(a) lipoprotein compared with diabetic controls without microalbuminuria.

Patients and methods

We recruited 41 patients, 20 males and 21 females with insulin dependent diabetes mellitus and microalbuminuria from our hospital's outpatient clinic on the basis of hospital records of microalbuminuria. All patients had had onset of diabetes before the age of 30. We defined microalbuminuria as albumin excretion $\geq 15 \mu\text{g}/\text{min}$ and $\leq 200 \mu\text{g}/\text{min}$ in at least two consecutive or two of three timed overnight urine samples. The urine samples did not show leucocyturia when tested by dipsticks. None of the patients had a history of non-diabetic renal disease, and none had ketoacidosis at the time of sampling.

The patients with microalbuminuria were compared with 35 insulin dependent diabetic patients without microalbuminuria. The sex ratio, age, duration of diabetes, and concentrations of haemoglobin A_{1c} (means of three values) were similar in both groups. Haemoglobin A_{1c} concentrations were measured over 12 to 15 months, starting from 12 to 28 months and

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ending from 0 to 23 months before the blood sampling for determination of lipid and apolipoprotein concentrations. The mean haemoglobin A_{1c} concentration was calculated by using the measurements taken during the inclusion period and that taken at the time of blood sampling for measuring lipoprotein concentrations. One person in the microalbuminuric group was being treated for hypertension with enalapril and frusemide, and one person in the control group was taking prazosin for hypertension. Four women in the microalbuminuric group and six women in the control group used combined oestrogen and gestagen contraceptive pills, and two women in the control group used gestagen contraceptive pills. One person in the microalbuminuric group took carbamazepine for epilepsy. Table I summarises the clinical details of the two groups.

The blood samples were drawn between 8 am and 10 am after an overnight fast and before insulin was taken. The samples were centrifuged within an hour and the plasma stored at -70°C .

Total serum cholesterol, high density lipoprotein cholesterol, and triglyceride concentrations were measured by enzymatic methods.^{23,24} Very low density lipoprotein cholesterol concentration was calculated by Friedewald's formula (very low density lipoprotein cholesterol = triglycerides $\times$ 0.45),²⁵ and low density lipoprotein cholesterol concentration was calculated by subtracting the sum of high density lipoprotein cholesterol and very low density lipoprotein cholesterol concentrations from the total cholesterol concentration. Apolipoprotein A-I and apolipoprotein B were measured by turbidimetry with antiserum and standard reagents from Behringwerke AG, Marburg. Apolipoprotein A-II was measured by radial immunodiffusion with antiserum from Boehringer Mannheim, Germany.

The albumin concentration in urine was measured by a immunoturbidimetric method. The variation coefficient in the range of 10-50 mg/l was 4.7%. Lp(a) lipoprotein was measured by quantitative immunoelectrophoresis with highly specific antiserum prepared at the Institute of Medical Genetics, University of Oslo, incorporated in the agarose gel. Reference human serum with a high concentration of Lp(a) lipoprotein was included at four dilutions in each electrophoresis run. The concentration of Lp(a) lipoprotein in the reference serum was determined by comparing precipitin "rockets" obtained with purified Lp(a) lipoprotein preparations whose protein concentrations had been determined by the micro-Kjeldahl method. Reproducibility was investigated by testing split serum samples blinded; this gave a correlation coefficient of 0.98.¹⁹ Concentration of Lp(a) lipoprotein is given as mg protein/100 ml as to calculate a molarity the protein result would have to be multiplied by a correction factor taking into account the relative

Serum Lp(a) lipoprotein concentrations in insulin dependent patients with and without microalbuminuria. Median value in each group depicted by horizontal line

amount of protein in Lp(a) lipoprotein, which varies among isoforms of the polypeptide chain carrying the Lp(a) antigen. All apolipoprotein concentrations are also given as mg/100 ml.

The Mann-Whitney U test was used to compare distributions. A value below 0.05 was taken as significant. Correlation between parameters was given as Pearson's r (least square method). The proportions of the groups having a Lp(a) lipoprotein value above the upper quartile were compared by using χ^2 test with Yates's correction.

Results

The median Lp(a) lipoprotein concentration in the microalbuminuric group (10.0 mg/100 ml) was significantly higher than that in the control group (4.9 mg/100 ml; $p=0.007$) (figure). The association between Lp(a) lipoprotein and cardiovascular disease is largely confined to patients with values above the upper quartile of the distribution of serum Lp(a) concentrations.¹⁹ In healthy unrelated individuals examined in the same laboratory with the same technique, the upper quartile was 13.55 mg/100 ml (data not shown). In our study 17 patients (41%) in the microalbuminuric group compared with five (14%) in control group had serum Lp(a) lipoprotein values above the upper quartile ($p=0.02$).

In the control group the haemoglobin A_{1c} concentration at the time of blood sampling and the mean concentration both showed a significant positive correlation with the serum Lp(a) lipoprotein concentration ($r=0.50$, $p=0.001$ and $r=0.35$, $p=0.02$, respectively). In the microalbuminuric group the correlations were not significant ($r=0.21$, $p=0.09$ at the time of blood sampling and $r=0.20$, $p=0.11$ for mean concentration).

The median concentrations of serum triglycerides and very low density lipoprotein cholesterol were significantly higher in the microalbuminuric group than in the control group (table II). Median serum concentrations of apolipoprotein A-II were identical in the two groups, and total cholesterol, high density lipoprotein cholesterol, low density lipoprotein

TABLE I—Clinical details of insulin dependent diabetic patients with and without microalbuminuria

	With microalbuminuria (n=41)	Without microalbuminuria (n=35)	p Value
Male/female	20/21	18/17	
Median (range) age (years)	19 (14-49)	20 (15-43)	0.16
Median (range) duration of diabetes (years)	13 (7-33)	14 (7-33)	0.44
Median (range) body mass index (kg/m ²)	23.8 (19.5-31.4)	23.8 (20.1-30.0)	0.29
Median (range) haemoglobin A _{1c} (%)	8.8 (6.3-14.5)	9.4 (7.0-11.2)	0.32
Median (range) mean haemoglobin A _{1c} (%)	9.4 (6.7-12.6)	9.0 (6.3-11.9)	0.08
Median (range) dose of insulin (IU/kg/day)	0.90 (0.49-1.28)	0.83 (0.40-1.28)	0.09
Median (range) mean albumin excretion rate (μg/min)*	30 (14-186)	5 (2-13)	<0.001

*In two patients some of the albumin excretion rates were given as mg/24h. As the albumin excretion rate is about 25% higher when measured over 24h rather than overnight the results were converted to μg/min and reduced by 25% to obtain comparable values.

TABLE II—Median (95% confidence interval) serum concentrations of lipids and lipoprotein in insulin dependent diabetic patients with and without microalbuminuria

	With microalbuminuria (n=41)	Without microalbuminuria (n=35)	p Value
Lp(a) lipoprotein (mg protein/ 100 ml)	10.0 (5.7 to 16.8)	4.9 (3.4 to 6.4)	0.007
Cholesterol (mmol/l)	5.3 (4.8 to 5.7)	4.9 (4.6 to 5.0)	0.11
Triglycerides (mmol/l)	1.15 (1.01 to 1.48)	0.88 (0.76 to 0.99)	0.03
High density lipoprotein cholesterol (mmol/l)	1.20 (1.05 to 1.32)	1.18 (1.10 to 1.29)	0.43
Very low density lipoprotein cholesterol (mmol/l)	0.52 (0.45 to 0.67)	0.40 (0.34 to 0.45)	0.03
Low density lipoprotein cholesterol (mmol/l)	3.54 (2.83 to 4.06)	3.11 (3.02 to 3.56)	0.18
Apolipoprotein A-I (mg protein/ 100 ml)	118 (109 to 124)	111 (105 to 119)	0.10
Apolipoprotein A-II (mg protein/ 100 ml)	41 (38 to 44)	41 (39 to 42)	0.28
Apolipoprotein B (mg protein/ 100 ml)	109 (95 to 119)	97 (81 to 106)	0.08

cholesterol, apolipoprotein A-I, and apolipoprotein B concentrations were not significantly higher in the microalbuminuric group (table II).

Discussion

We have shown that insulin dependent diabetic patients with microalbuminuria have a significantly higher concentration of Lp(a) lipoprotein than those without microalbuminuria of the same sex, age, duration of diabetes, and haemoglobin A_{1c} concentration. This difference cannot be explained by differences in blood glucose control as the haemoglobin A_{1c} concentration was similar in the two groups.

Significantly more microalbuminuric patients than control patients had Lp(a) lipoprotein concentrations above the upper quartile of the normal distribution. The number was higher than expected in the microalbuminuric group and lower than expected in the control group. As an increased plasma concentration of Lp(a) lipoprotein is strongly correlated with the risk for coronary heart disease, finding a higher value in microalbuminuric diabetic patients may be important in understanding the increased prevalence of cardiovascular disease in diabetic patients with nephropathy. It is remarkable that Lp(a) lipoprotein concentrations were so different in the two groups of young insulin dependent diabetic patients without any clinical signs of arteriosclerotic disease, that differed only in the excretion of small amounts of urinary albumin.

Serum Lp(a) lipoprotein concentrations vary 1000-fold among individuals, and the distribution is skewed in Caucasian populations, with a peak in the low range and a tail of higher values.^{15,26,27} The serum concentration is independent of age and sex, and it is not influenced by lipid lowering diets.²⁸ However, recent investigations have shown a correlation between Lp(a) lipoprotein concentrations and parameters of glycaemic control in insulin dependent diabetic patients.^{29,30} We found a significant correlation between Lp(a) lipoprotein and haemoglobin A_{1c} concentration only in the patients without microalbuminuria. Lp(a) lipoprotein can accumulate in artery walls,^{21,31} and Lp(a) apolipoprotein competes with plasminogen for the binding sites on the endothelial cell surface.³² Lp(a) lipoprotein has been shown to bind tissue type plasminogen activator in vitro, thus inhibiting activation of plasminogen.³³ Lp(a) lipoprotein may therefore contribute to the development of atherothrombotic disease by inhibiting fibrinolysis.

The fibrinolytic system is changed in diabetic patients with microalbuminuria.⁶ Increased concentrations of Lp(a) lipoprotein might bind more tissue-type plasminogen activator leading to less release of this factor into the blood as described in insulin dependent diabetic patients with microalbuminuria.⁶

The serum concentrations of triglycerides and very

low density lipoprotein cholesterol were significantly higher in the microalbuminuric patients. The very low density lipoprotein cholesterol concentrations are in accordance with the findings of others, while the difference in triglyceride concentration between the groups is somewhat greater in our series.^{4,5}

Only about 35% of patients with insulin dependent diabetes develop nephropathy.³⁴ Glycaemic control is a crucial determinant for the development of nephropathy,^{35,37} but there also is a genetic factor.³⁸ As the serum concentration of Lp(a) lipoprotein is genetically determined in healthy populations Lp(a) lipoprotein may be associated with the genetic predisposition to diabetic nephropathy.

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Total cholesterol, low density lipoprotein cholesterol, and high density lipoprotein cholesterol and coronary heart disease in Scotland

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Abstract

Objective—To investigate long term changes in total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol concentrations and in measures of other risk factors for coronary heart disease and to assess their importance for the development of coronary heart disease in Scottish men.

Design—Longitudinal study entailing follow up in 1988-9 of men investigated during a study in 1976.

Setting—Edinburgh, Scotland.

Subjects—107 men from Edinburgh who had taken part in a comparative study of risk factors for heart disease with Swedish men in 1976 when aged 40.

Intervention—The men were invited to attend a follow up clinic in 1988-9 for measurement of cholesterol concentrations and other risk factor measurements. Eighty three attended and 24 refused to or could not attend.

Main outcome measures—Changes in total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol concentrations, body weight, weight to height index, prevalence of smoking, and alcohol intake; number of coronary artery disease events.

Results—Mean serum total cholesterol concentration increased over the 12 years mainly due to an increase in the low density lipoprotein cholesterol fraction (from 3.53 (SD 0.09) to 4.56 (0.11) mmol/l) despite a reduction in high density lipoprotein cholesterol concentration. Body weight and weight to height index increased. Fewer men smoked more than 15 cigarettes/day in 1988-9 than in 1976. Blood pressure remained stable and fasting triglyceride concentrations did not change. The frequency of corneal arcus doubled. Alcohol consumption decreased significantly. Eleven men developed clinical coronary heart disease. High low density lipoprotein and low high density lipoprotein cholesterol concentrations in 1976, but not total cholesterol concentration, significantly predicted coronary heart disease ($p=0.05$). Almost all of the men who developed coronary heart disease were smokers (91% v 53%, $p<0.05$).

Conclusion—Over 12 years the lipid profile deteriorated significantly in this healthy cohort of young men. Smoking, a low high density lipoprotein concentration and a raised low density lipoprotein concentration were all associated with coronary heart disease in middle aged Scottish men, whereas

there was no association for total cholesterol concentration. The findings have implications for screening programmes.

Introduction

Mortality from coronary heart disease is not uniform in Europe, either between countries or even within a single country. In Britain,^{1,2} Scotland and Northern Ireland have the highest mortality from coronary heart disease in middle aged men. The factors causing this geographic variation are not fully understood. Regional differences in standardised mortality ratios for coronary heart disease in Scotland³ and in Britain⁴ cannot be explained by variations in the average total cholesterol or high density lipoprotein cholesterol concentration, although variations in smoking and blood pressure provide a partial explanation.

In 1976 a study in Edinburgh and Stockholm gathered cross sectional data and explored reasons for the threefold excess of mortality from coronary heart disease in men aged 40 from Edinburgh.⁵ The men from Edinburgh smoked more cigarettes, had slightly higher blood pressure, were more obese, had a greater insulin response to an oral glucose tolerance test, and had significantly lower adipose linoleic acid concentrations than men aged 40 from Stockholm. In addition they had higher fasting triglyceride and lower high density lipoprotein concentrations.⁵ Serum total cholesterol and low density lipoprotein cholesterol concentrations did not differ between men from the two cities. This cross sectional study suggested, therefore, that raised cholesterol concentration may not be a major contributor to the high mortality from coronary heart disease in Scotland.

In assessing coronary risk factors it is also important to collect longitudinal data. Prospective studies have identified the increased risk of coronary disease associated with raised cholesterol concentration.^{6,7} The importance of a low high density lipoprotein cholesterol concentration has been controversial.⁸⁻¹⁰ In Britain, the regional heart study initially reported the lack of an independent association between a low high density lipoprotein cholesterol and mortality from coronary heart disease,¹¹ but subsequent reports suggest a different interpretation.¹²

We examined whether total cholesterol, high density lipoprotein cholesterol, and low density lipoprotein cholesterol concentrations measured in the 1976 study proved to be significant factors in predicting those men

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