Limited discriminant value of lipoprotein AI, lipoprotein Lp(a) and other lipoprotein particles in patients with and without early onset ischaemic heart disease

D T Vallance, H A Staunton, A F Winder

Abstract

Aims—To assess whether the ability of lipoprotein related variables to discriminate between individuals with or without premature clinical ischaemic heart disease (IHD) was improved using data on high density lipoprotein–lipoprotein AI (HDL–LpAI) fractions, alone or in combination with data on Lp(a).

Methods—Lipid and apolipoprotein concentrations were measured in 26 middle-aged men (mean age 50.3 years) with early onset IHD and coronary artery bypass grafting prior to sampling, and in 26 matched lipaemic and 26 normolipaeamic asymptomatic controls.

Results—Triglyceride and Lp(a) concentrations were higher, while HDL cholesterol and apolipoprotein A-I (apoA-I) concentrations were lower in patients than in controls. LpAI concentrations were also lower in IHD patients and were correlated with HDL and apoA-I in both IHD and control groups. Lp(a) was not correlated with any other lipid or apolipoprotein measured in either patients or controls. Univariate discriminant function analysis showed that the proportion correctly classified as patients or controls was marginally greater using LpAI concentrations as the discriminator, which was not increased in combination with Lp(a). Serum triglycerides, HDL cholesterol, apoA-I and Lp(a) alone all had similar, but weaker, discriminant power, which increased in various combinations with LpAI.

Conclusions—LpAI particle measurement may be useful in research to define mechanisms of cardiovascular protection by HDL but the discriminating power for IHD was only marginally superior to measuring total apoA-I or Lp(a) concentrations. Little further advantage arose through combining LpAI data with other variables.

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Serum cholesterol is a risk factor for ischaemic heart disease (IHD) from population and group analysis, but has a low predic-

tive power for the development of premature IHD. Other lipid risk factors have been applied in an attempt to improve that correlation. These include low (LDL) and high density lipoprotein (HDL) cholesterol, offering little benefit over total cholesterol,¹ apolipoprotein A-I (apoA-I),² apoB,^{3,4} both apoA-I and apoB,⁵ and lipoprotein(a) (Lp(a)).⁶⁻⁸ HDL subfractions of different lipid content and density (HDL2 and HDL3) and of variable apolipoprotein content have also been examined as these various fractions may have different metabolic roles and thus associations with IHD. There are at least two subpopulations of HDL which contain apoA-I: those particles containing apoA-I in association with apoA-II (LpAI:AI) and those containing apoA-I only (LpAI).⁹⁻¹⁴ These two particles are affected differently by drugs¹⁵ and alcohol,¹⁶ which is consistent with their different metabolic roles and clinical importance. LpAI may act via apoA-I to facilitate cholesterol trafficking between intracellular and plasma membrane pools in the promotion of cholesterol efflux.^{17,18} Below-average levels of LpAI particles were reported in patients with established IHD¹⁹ although the association has not been consistently supported in further studies,²⁰ in which concomitant cigarette smoking and use of cardioprotective, antihypertensive or lipid modifying medication with potential effects on HDL were potentially confounding.

A study of middle-aged men after myocardial infarction reported that Lp(a) concentrations were as good as family history at discriminating between patients and controls²¹; other authors have reported that plasma Lp(a) concentrations above 30 mg/dl are associated with a twofold increase in the risk of premature IHD.²² Lp(a) was also the strongest predictor of angiographically documented IHD in individuals with heterozygous familial hypercholesterolaemia and increased LDL cholesterol concentrations,⁷ an association supported by some⁸ but not all later studies.²³

We have studied a group of patients with premature IHD, plus matched controls, to determine whether any differences in apoA-I concentrations were associated with similar differences in LpAI concentrations, and whether LpAI was a better marker of coronary risk than total apoA-I. We have also measured Lp(a) concentrations to see

Department of Chemical Pathology and Human Metabolism, Royal Free Hospital School of Medicine, University of London, Pond Street, London NW3 2QG
D T Vallance
A F Winder

Department of Cardiological Sciences, St George's Hospital School of Medicine, University of London, London
H A Staunton

Correspondence to: Professor A F Winder.
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whether discrimination between those with and without IHD could be improved by measuring Lp(a) in addition to apoA-I and LpAI.

Methods

Twenty six middle-aged men, aged from 41 to 55 years (mean 50.3 years), were selected from a well documented register of individuals established to identify underlying genetic factors in families with a history of early onset IHD, but which turned out to reveal the widespread occurrence of unrecognised dyslipoproteinaemia.^{24 25} All had undergone coronary artery bypass grafting (CABG) when aged less than 55 years. Venous blood samples were taken at least six months after CABG to exclude any temporary effects of surgery on lipoprotein profiles. Participants were selected from the major survey because they were not taking any cardioprotective, antihypertensive or lipid modifying drugs and had either never smoked or had not smoked for at least one year before sampling. Patients with known familial hypercholesterolaemia, familial (type III) dysbeta-lipoproteinaemia, diabetes, thyroid, renal, or hepatic disorders were excluded from the study. The validity of these exclusions was supported by further laboratory studies including apo-E phenotyping and clinical examination including exclusion of familial hypercholesterolaemia through application of the criteria of the Simon Broome familial hypercholesterolaemia register.²⁶

Assessment of the predictive power of HDL related and other recorded variables was attempted through comparisons with two matched control groups, each assembled from 26 appropriate individuals who similarly were not taking any potentially confounding medication. Any former cigarette smokers had not smoked for at least one year. Data were assembled from 26 lipaemic but asymptomatic men who also had normal electrocardiograms, 18 who were taking part in the same family study as for the IHD group, and eight who were patients with similar total plasma cholesterol concentrations attending our lipid clinic over the same period (control group 1, lipaemic controls). The controls were derived from similar social groups and matched with the IHD group for age, body mass index, blood pressure, and plasma cholesterol concentrations (table 1), with exclusion criteria including underlying disorders as for the IHD group. Data were also assembled on a second group of 26 male normal but sedentary volunteers about to take part in a study of exercise and cardiovascular responses,²⁷ selected to be matched with the IHD group for age, body mass index and diastolic blood pressure (but not total or HDL cholesterol or systolic blood pressure) and with samples analysed over the same period (control group 2, exercise controls). Declared alcohol consumption was similar for both control and patient groups: prospective participants with declared consumption over 20 units per week were excluded.

Total cholesterol, HDL cholesterol and

triglyceride concentrations were determined on serum from venous blood samples delivered to the laboratory within 24 hours of venepuncture, and separated on arrival. Samples for apoA-I, apoB, Lp(a), and LpAI particles were stored at -70°C for up to six months and defrosted at 37°C immediately before analysis. Total cholesterol and triglyceride concentrations were measured using enzymatic methods (Boehringer Mannheim, Sussex, England), supernatant HDL cholesterol was measured following precipitation using a heparin-manganese method,²⁸ all with quality assurance from national schemes, and LDL cholesterol was calculated using the Friedewald formula except in two patients with plasma triglyceride concentrations exceeding 4.5 mmol/l, invalidating this calculation.²⁹ Apoproteins were measured using immunoturbidimetry (Incstar, Berks, England). Lp(a) was measured using an enzyme-linked immunosorbent assay (ELISA) technique (Immuno, Kent, England) with support from a national multicentre quality control scheme. LpAI particles were measured using an electroimmunoassay method developed and validated by Parra *et al*,³⁰ using pre-prepared agarose plates (Labmedics, Manchester, England) evaluated against lyophilised control serum (Boehringer Mannheim Precinorm) and an internal frozen pooled serum sample stored at -70°C . Between run coefficients of variation were 4.4% at a mean of 0.46 g/l and 3.7% at a mean of 0.58 g/l.

Statistical analyses were performed using unpaired *t* tests, Spearman correlation coefficients, and by univariate discriminant function analysis. Multivariate analysis was inappropriate partly because of the small numbers of participants but mainly because the primary interest was in the strength of associations with LpAI, in comparison with other variables, and LpAI is not independent of HDL or other HDL related variables including apoA-I and triglycerides. Triglyceride and Lp(a) concentrations were normalised by logarithmic transformation before statistical analysis.

Results

Clinical and biochemical characteristics of patients with IHD and matched lipaemic and exercise controls are given in tables 1 and 2. The exercise control group was not matched

Table 1 Clinical characteristics (mean $\pm$ SD) of IHD and control groups (lipaemic men without IHD and normal sedentary men (exercise controls))

Variable	IHD	Lipaemic controls	Exercise controls
Age (years)	50.3 $\pm$ 6.5	51.6 $\pm$ 6.1	51.0 $\pm$ 5.9
BMI (kg/m ²)	26.1 $\pm$ 3.8	25.6 $\pm$ 3.4	26.2 $\pm$ 3.2
Blood pressure (mmHg)			
systolic	132 $\pm$ 16	129 $\pm$ 12	121 $\pm$ 13*
diastolic	82 $\pm$ 10	80 $\pm$ 9	82 $\pm$ 9

* *p* < 0.05

BMI = body mass index.

Table 2 Lipid and lipoprotein concentrations (mean $\pm$ SD, except $\S$ median and range) in IHD and control groups (lipaemic men without IHD and normal sedentary men (exercise controls))

Variable	IHD	Lipaemic controls	Exercise controls
Total cholesterol (mmol/l)	7.1 $\pm$ 1.3	7.0 $\pm$ 0.8	6.1 $\pm$ 1.0†
LDL cholesterol (mmol/l)	4.9 $\pm$ 1.4	4.8 $\pm$ 0.7	3.9 $\pm$ 0.9†
HDL cholesterol (mmol/l)	1.1 $\pm$ 0.4	1.4 $\pm$ 0.3†	1.4 $\pm$ 0.3†
Triglyceride (mmol/l)§	2.3 (0.8–4.9)	1.7 (0.6–3.7)	1.8 (0.8–4.6)
Log (triglyceride)	0.32 $\pm$ 0.23	0.19 $\pm$ 0.18*	0.20 $\pm$ 0.19*
Apo A-I (g/l)	1.29 $\pm$ 0.24	1.47 $\pm$ 0.25†	1.41 $\pm$ 0.20†
Apo B-100 (g/l)	0.92 $\pm$ 0.23	0.86 $\pm$ 0.16	0.78 $\pm$ 0.18*
Lp(a) (mg/dl)§	26 (5–57)	15 (5–67)	14 (5–79)
Log[Lp(a)]	1.37 $\pm$ 0.24	1.15 $\pm$ 0.34*	1.09 $\pm$ 0.42*
LpAI (g/l)	0.45 $\pm$ 0.07	0.55 $\pm$ 0.09‡	0.52 $\pm$ 0.10‡

* $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$.

with the other groups for systolic blood pressure, total and LDL cholesterol and apoB-100. Otherwise, the IHD group had higher triglyceride ($p < 0.05$) and Lp(a) (log Lp(a), $p < 0.05$) concentrations than those in both control groups, while concentrations of HDL cholesterol ($p < 0.01$) and the HDL related variables apoA-I ($p < 0.05$) and LpAI ($p < 0.001$) were both significantly lower in patients than in controls. LpAI was correlated ($p < 0.05$) with HDL and apoA-I in the IHD and both control groups (table 3), while Lp(a) was not significantly correlated with any other lipid or apoprotein variable measured in the IHD or either control group (table 4).

Univariate discriminant function analysis of the IHD and lipaemic control group showed that the proportion of the study group correctly classified as having or not having clinical IHD was greatest using LpAI concentration as the single discriminator (table 5). Serum triglycerides, HDL cholesterol, apoA-I, and Lp(a) all had similar but marginally less discriminant power. Combining LpAI with apoA-I increased the

Table 3 Spearman correlation coefficients of LpAI particles with other risk factors in IHD and control groups (lipaemic men without IHD and normal sedentary men (exercise controls))

Variable	IHD	Lipaemic controls	Exercise controls
Total cholesterol	0.245	0.176	0.068
Triglycerides	-0.131	-0.222	-0.192
HDL cholesterol	0.715*	0.596*	0.502*
ApoA-I	0.682*	0.528*	0.441*
ApoB-100	0.175	0.142	-0.074
Lp(a)	0.013	-0.061	-0.018

* $p < 0.05$.

Table 4 Spearman correlation coefficients of Lp(a) with other risk factors in IHD and control groups (lipaemic men without IHD and normal sedentary men (exercise controls))

Variable	IHD	Lipaemic controls	Exercise controls
Cholesterol	0.196	-0.112	-0.050
Triglycerides	-0.243	-0.070	-0.081
HDL	0.208	0.155	0.111
ApoA-I	0.110	-0.018	-0.088
ApoB	0.082	-0.033	-0.117
LpAI	0.013	-0.061	-0.018

Table 5 Discriminant analysis of lipid risk factors

Variable	Proportion correctly classified (%)			
	P + C1	P + C2	P + C1 + C2	
Total cholesterol	50.0	71.3	59.0	
HDL cholesterol	64.5	65.4	65.2	
Triglycerides	62.8	61.5	62.8	
LpAI particles	69.2	66.9	68.1	
Apo A-I	64.0	65.5	66.7	
Apo B-100	59.6	65.8	63.1	
Lp(a)	63.5	60.4	61.5	
LpAI + apo A-I	75.0	64.6	67.4	
LpAI + TG	78.8	61.8	65.4	
LpAI + Lp(a)	69.2	61.5	62.5	
LpAI + apo A-I + TG	80.8	68.3	76.5	
LpAI + apo A-I + TG + Lp(a)	78.8	71.2	76.9	

P = probands with IHD; C1 = lipaemic controls without IHD; C2 = normolipaemic exercise controls without IHD; TG = triglycerides.

proportion correctly classified, which was further improved by including triglycerides. However, adding Lp(a) to the variables LpAI, apoA-I, and triglycerides did not produce any further improvement (table 5). The exercise normolipaemic control group was not matched for cholesterol or the dependent variables LDL cholesterol and apoB-100. Results for the HDL dependent variables were as for the lipaemic control group, LpAI being marginally the most powerful discriminator, a similar result arising when the two control groups were combined (table 5).

Discussion

LpAI particles, accounting for approximately 40% of total apoA-I,³¹ have been proposed as the antiatherogenic fraction of HDL¹⁹ and might therefore be a better index of coronary risk than either HDL, apoA-I or HDL₂. The HDL₂ fraction is rich in LpAI, and a study in which any effects of smoking habits and medication were not excluded also suggested that the apoA-I component of LpAI particles was preferentially reduced in patients with low HDL concentrations.²⁰ Although concomitant cigarette smoking and beta blocker medication may not have specific effects on the metabolism of HDL and its components,^{32,33} these potential confounding effects and the different effects of short term and chronic alcohol abuse³⁴ have been specifically excluded through selection of patient and control groups. Diets were not specifically controlled: diets high in polyunsaturated fatty acids decrease LpAI concentrations compared with diets high in saturated fatty acids.³⁵ Results of the present study, showing that the concentrations of LpAI particles are reduced in patients with early onset IHD, are in agreement with other studies reporting—for example, decreased LpAI concentrations in patients with angiographically proven coronary artery disease,¹⁹ in male survivors of myocardial infarction^{4,5} and in patients studied before coronary artery bypass surgery.³⁶ Discrimination in some of these earlier studies was marginally improved by additional weighting, incorporating other lipoprotein related variables. Lp(a) concentrations were noticeably higher in our IHD group compared

with controls but the proportion of individuals correctly classified was not greatly improved by adding Lp(a) to the LpAI data, or in other combinations (LpAI, apoA-I and triglycerides). Lp(a) concentrations did not correlate with LpAI or other HDL related or lipoprotein variables, such as total cholesterol and apoB-100, again emphasising that there are some differences in the regulatory mechanisms which control the synthesis and catabolism of LDL and those which control Lp(a), even though both contain apoB-100. Some but not all cross sectional studies in familial hypercholesterolaemia and less specific dyslipidaemic groups have reported that participants with IHD have higher serum concentrations than those without IHD.^{7 8 23 35}

The number of patients and controls in the present study is small, but participant selection and the criteria for matching have advantages with respect to confounding variables, notably smoking and medication. All patients with IHD had undergone coronary angiography and CABG at least six months before sampling and had confirmed disease. Some participants in the control groups may have had significant asymptomatic IHD, and their inclusion would weaken the discriminating power of the lipoprotein variables measured. However, all were asymptomatic, blood pressure had been assessed, some had undergone further cardiovascular assessment including resting electrocardiogram, and the normolipidaemic controls completed the exercise programme without developing symptoms of IHD.²⁷ Comparisons of this nature are the essence of the clinical problem addressed here and for which the assessment of additional laboratory variables could be helpful. LpAI:AI particles were not measured in our study. Reduced concentrations have been reported in hypertriglyceridaemic but not in normotriglyceridaemic patients with angiographically defined coronary artery disease, a discrepancy that may be because of reduced formation of LpAI:AI particles during lipolysis in hypertriglyceridaemic subjects.³³ Observations in transgenic animals³⁷ and the identification of human families lacking apoA-II but without premature coronary artery disease³⁸ suggest that apoA-I has a key role in IHD.

The results obtained here support earlier studies showing that LpAI concentrations are reduced in patients with early onset IHD. This association was also present in the absence of cigarette smoking and cardioprotective, antihypertensive or lipid modulating medication. As in earlier studies, the discriminant power of LpAI strengthened when various other lipoprotein variables were used in combination with LpAI. Discriminant power was not increased by the incorporation of Lp(a), and LpAI alone offered only marginal advantage over the established markers total HDL and apoA-I.

Lipoprotein variables are only one aspect of a range of associations with premature IHD. Subfractions within the HDL particle range are of great interest in research to identify the

precise mechanisms by which HDL may exert protective effects against atherosclerosis. At present, as with Lp(a), LpAI measurement should not be used alone or prevail over the range lipid and apolipoprotein tests currently available for assessing individual IHD risk.

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