High prevalence of hypertriglyceridaemia and apolipoprotein abnormalities in coronary artery disease

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SUMMARY   Serum lipids and apolipoproteins A-I and B were measured in 174 men aged < 60 with angiographically confirmed coronary artery disease and in 572 healthy control men. Two thirds of the patients had raised age-corrected values of fasting serum cholesterol and/or triglyceride and/or a low high density lipoprotein (HDL) cholesterol compared with the controls. Eighteen (30%) of the 61 normolipidaemic patients had a concentration of serum apolipoprotein A-I below the 5th percentile of 233 controls. In normolipidaemic patients on β blockers the relative prevalence of serum low density lipoprotein (LDL)-apolipoprotein B values above the 95th percentile of 339 controls was significantly increased. Discriminant function analysis showed that a raised concentration of serum triglyceride was the best discriminant between patients and controls, with raised LDL-apolipoprotein B and reduced apolipoprotein A-I coming second only to triglyceride in analyses where each was separately compared with all the lipid variables. These associations were highly significant and were independent of other influences, including β blockade.

These findings re-emphasise the importance of hypertriglyceridaemia as a risk factor and confirm that apolipoprotein abnormalities occur frequently in coronary disease, even in normolipidaemic patients.

Evidence that serum apolipoprotein measurements are useful indices of the risk of coronary heart disease was reviewed four years ago[1 2] and several reports have since appeared which further substantiate this concept.[3-9] Opinions are divided, however, as to whether increases in apolipoprotein B, the protein moiety of low density lipoprotein (LDL), or decreases in apolipoprotein A-I, the major protein of high density lipoprotein (HDL), correlate any better with angiographically defined coronary artery disease than do increases in LDL cholesterol or decreases in HDL cholesterol.[6-9]

The object of the present study was to assess the value of apolipoprotein measurements as risk factors by investigating the frequency of serum lipid abnormalities in medically treated patients with angiographically confirmed coronary artery disease and by determining how often apolipoprotein abnormalities

occurred, especially in those patients found to be normolipidaemic.

Patients and methods

During the period June 1985–July 1986 all men below the age of 60 admitted to Hammersmith Hospital to undergo diagnostic coronary angiography or coronary artery bypass grafting had blood taken on the morning before these procedures, after an overnight fast, for measurement of serum lipids and apolipoproteins. Patients with diabetes, those on lipid lowering agents, and those who had already undergone coronary artery bypass grafting or had had a myocardial infarct within the preceding two months were excluded, as were those in whom the angiogram did not show significant coronary artery disease, defined as luminal stenosis of > 50% in at least one vessel. Concentrations of serum total cholesterol and triglyceride were analysed enzymatically (Technicon Auto Analyzer methods SE4-0039 PE and SE-0065 FBI), as was serum HDL cholesterol after precipitation with heparin-manga-

nese.[10] The concentration of LDL cholesterol was calculated by the formula of Friedewald et al[11] except in patients with triglyceride values of 4·5 mmol/l, in whom LDL cholesterol was measured after ultra-centrifugation of serum.[12] Serum apolipoprotein A-I was measured by an immunoturbidometric method (Orion Diagnostica, marketed in the United Kingdom by Oxoid, Basingstoke, Hampshire) and LDL-apolipoprotein B by radial immunodiffusion, as originally described by Sniderman et al[13] and modified by Havekes et al.[14] Results were complete for 174 of the 188 consecutive patients who originally qualified for this survey.

Control values for fasting serum lipids and apolipoproteins were derived from symptom free men aged 20–60 with normal electrocardiograms who had undergone health screening at the British United Provident Association Medical Centre (BUPA), London between March 1983 and July 1986. Serum was obtained from 339 men for determination of LDL-apolipoprotein B and subsequently from another 233 men for determination of apolipoprotein A-I. Measurement of LDL-apolipoprotein B in patients with coronary artery disease and in controls was always performed by IT and apolipoprotein A-I was measured by DW. In all the controls, enzymatic analysis of total cholesterol, triglyceride, and HDL cholesterol (the last after dextran sulphate-magnesium precipitation)[15] was performed by JS Pathology Services on a Technicon SMAC System similar to that used at Hammersmith Hospital. To correct for interlaboratory differences multiple split sample assays were performed over a wide range of concentrations in each laboratory and conversion factors derived from these comparisons were used to make the Hammersmith Hospital values compatible with JS Pathology Services values.

There were no significant differences between the two control groups—their mean values of age, serum lipids, and lipoproteins being almost identical (table 1). These were therefore combined into one control group of 572 men for all case-control comparisons except those for apolipoprotein values. Because the patients with coronary artery disease were on average older than the controls (mean (SD) age of patients 50·4 (6·7) v mean age of controls 43·9 (8·5)) all lipid, lipoprotein, and apolipoprotein values were adjusted to the age of 45 by linear regressions techniques. Triglyceride values were log transformed before statistical analysis but untransformed values are given in the text and tables.

Statistical comparisons were undertaken with the aid of the NWA StatPak (Multifunction Statistics Library, Version 3.1, Northwest Analytical, Portland, Oregon) and SPSS/PC+ (SPSS, Chicago, Illinois). Discriminant function analysis was perfor-

med with BMDP statistical software (University of California Press, Berkeley, California).

Results

In the 174 patients with coronary artery disease the mean values of total cholesterol, triglyceride, and LDL cholesterol were higher, and those of HDL cholesterol were lower, than in the 572 controls (p < 0·01 for each pair of variables) (table 2). Apolipoprotein A-I values were lower and LDL-apolipoprotein B values were higher in patients with coronary artery disease than in their respective subgroups of controls (p < 0·001 in each instance). Figures 1–4 show the frequency distributions of concentrations of serum lipids and lipoproteins in patients with coronary artery disease and in controls; the distribution of triglycerides was positively skewed. High values of total cholesterol, triglyceride, and LDL cholesterol and low values of HDL cholesterol were more common in patients than in controls.

Figures 5 and 6 show the frequency distributions of the values for serum apolipoprotein A-I and LDL-apolipoprotein B in patients with coronary artery disease and the corresponding controls. Patients with coronary artery disease had a higher frequency of

Table 1 *Serum lipids in the two subgroups of controls (mean (SD))*

Variable	Apo A-I controls (n = 233)	Apo B controls (n = 339)
Age (yr)	44 (8)	44 (9)
Total cholesterol (mmol/l)	6·16 (1·10)	6·13 (1·05)
Triglyceride (mmol/l)	1·40 (0·74)	1·39 (0·70)
HDL cholesterol (mmol/l)	1·25 (0·30)	1·21 (0·29)
LDL cholesterol (mmol/l)	4·27 (1·05)	4·29 (1·00)

Apo A-I, apolipoprotein A-I; Apo B, apolipoprotein B.

Table 2 *Concentrations (mean (SD)) of serum lipid, lipoprotein, and apoprotein in patients with coronary artery disease and in controls*

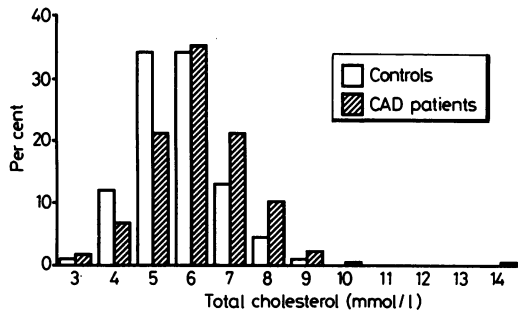| Variable	Patients (n = 174)	Controls (n = 572)	t	p
Total cholesterol (mmol/l)	6·66 (1·34)	6·14 (1·07)	5·27	<0·001
Triglyceride (mmol/l)	2·43 (1·37)	1·40 (0·71)	—	—
Log₁₀ triglyceride (mmol/l)	0·34 (0·20)	0·10 (0·20)	13·56	<0·001
HDL cholesterol (mmol/l)	0·98 (0·29)	1·22 (0·29)	−9·70	<0·001
LDL cholesterol (mmol/l)	4·54 (1·05)	4·28 (1·01)	2·87	<0·004
Apo A-I (mg/dl)	126 (23)	145 (22)*	−8·49	<0·001
LDL-apo B (mg/dl)	137 (38)	113 (26)†	8·26	<0·001

*n = 233; †n = 339.

Fig 1 *Frequency distributions of serum total cholesterol in controls (n = 572) and patients with coronary artery disease (CAD) (n = 174).*
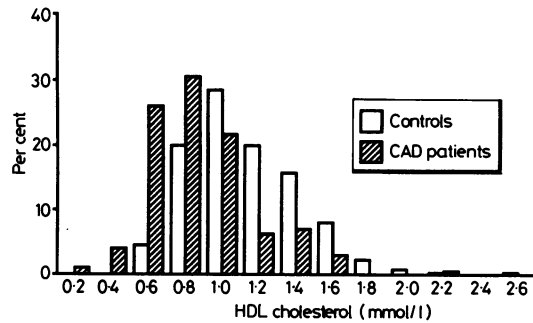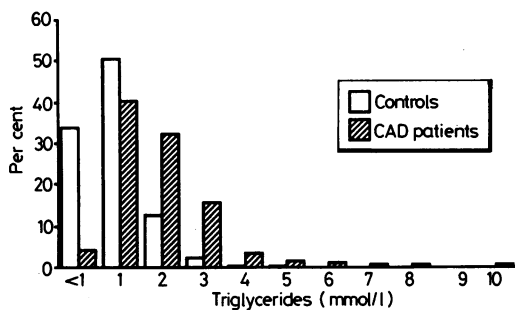

Fig 2 *Frequency distributions of serum triglyceride in controls and patients with coronary artery disease.*

Fig 3 *Frequency distributions of LDL cholesterol in controls and patients with coronary artery disease.*

Fig 4 *Frequency distributions of HDL cholesterol in controls and patients with coronary artery disease.*
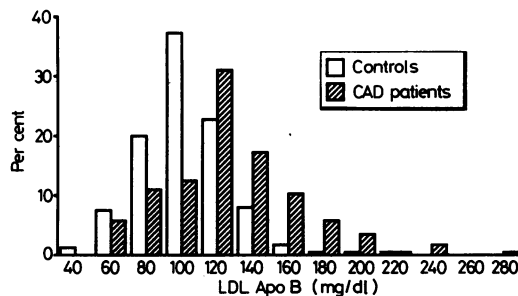

Fig 5 *Frequency distributions of LDL-apolipoprotein B in controls (n = 339) and patients with coronary artery disease.*
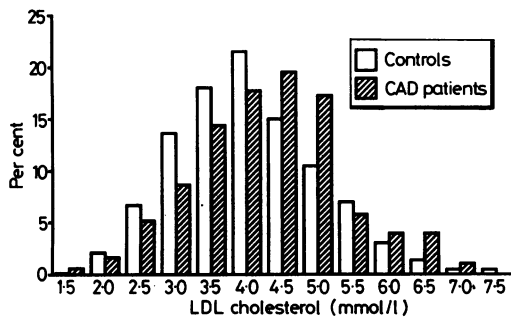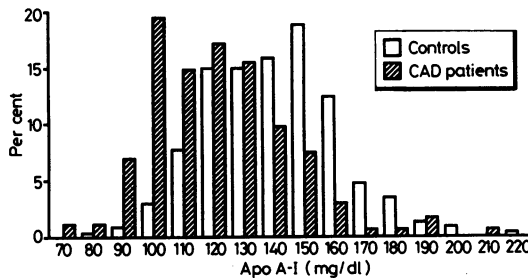

Fig 6 *Frequency distributions of apolipoprotein A-I in controls (n = 233) and patients with coronary artery disease.*

high LDL-apolipoprotein B values and low apolipoprotein A-I values than the controls, with less overlap between the two groups than was evident for LDL and HDL cholesterol (figs 3 and 4).

Table 3 shows the proportion of patients and controls with abnormal values of each measurement. For total cholesterol we used a cut off value of 6·7 mmol/l, which was the arbitrary upper limit of normal at the BUPA Medical Centre. For all the

other variables, the 95th percentile or, for HDL cholesterol and apolipoprotein A-I, the 5th percentile values of the controls were used to define abnormality. The final column of this table gives the ratio or relative prevalence of an abnormal value in patients with coronary artery disease compared with controls and the 95% confidence intervals. Relative prevalence values were lowest with total cholesterol (1·59) and LDL cholesterol (1·64); LDL cholesterol

Table 3 *Proportions of abnormal values in patients and controls*

Value	Patients (%)	Controls (%)	Relative prevalence (95% confidence interval)
Total cholesterol > 6·7 mmol/l	42·0	26·4	1·59* (1·27–1·99)
Triglyceride > 2·78 mmol/l	29·9	4·9	6·11* (3·99–9·36)
HDL cholesterol < 0·80 mmol/l	31·0	4·9	6·34* (4·15–9·69)
LDL cholesterol > 6·05 mmol/l	8·0	4·9	1·64 (0·88–3·04)
Apolipoprotein A-I < 110 mg/dl	29·3	4·7	6·21* (3·34–11·56)
LDL apolipoprotein B > 153 mg/dl	26·4	4·7	5·60* (3·27–9·60)

*Relative prevalence differs significantly from unity (p < 0·001).

did not differ significantly from unity. In contrast, abnormal values of triglyceride, HDL cholesterol, and each of the apolipoproteins were approximately six times more common in patients than in controls (p < 0·001 in each instance).

FREQUENCY OF ABNORMAL APOLIPOPROTEIN
VALUES IN NORMOLIPIDAEMIC PATIENTS

In those individuals with both normal total cholesterol and normal triglyceride (48% of the patients and 71% of controls) the relative prevalence of a low HDL cholesterol was 5·96 (95% confidence interval 3·35 − 10·61). None of these subjects had a raised LDL cholesterol value. Thirty five per cent of the patients with coronary artery disease and 68% of controls were normolipidaemic—that is they had normal concentrations of total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol. Table 4 shows the relative prevalence of apolipoprotein abnormalities in these normolipidaemic individuals. The relative prevalence of a low apolipoprotein A-I was significantly increased whereas that of a raised LDL-apolipoprotein B just failed to reach significance. The relative prevalence of a raised concentration of LDL-apolipoprotein B seemed to be higher in type IV individuals (raised triglyceride and normal LDL cholesterol) at 5·32 (0·78 − 36·35), but again this increase was not statistically significant.

CLINICAL CHARACTERISTICS OF PATIENTS WITH
CORONARY ARTERY DISEASE

Serum lipid and apolipoprotein values in patients with coronary artery disease were similar irrespective of whether patients were admitted for angiography or coronary artery bypass grafting, presence or absence of previous myocardial infarction, and whether or not they were European. Table 5 shows the frequency of these and other characteristics. The lack

Table 4 *Proportions of abnormal apoprotein values in normolipidaemic patients and controls*

Variable	Patients (%)	Controls (%)	Relative prevalence (95% confidence interval)
Apolipoprotein A-I < 110 mg/dl	29·5	5·6	5·31* (2·52–11·17)
LDL apolipoprotein B > 153 mg/dl	6·6	1·8	3·69 (0·95–14·33)

*Relative prevalence differs significantly from unity (p < 0·001).

Table 5 *Clinical characteristics of patients*

Characteristic	No	%
Admitted for CABG	47	27
Previous MI	103	59
European	140	80
On β blockers	113	65
Family history of CHD	99	57
Cigarette smoking	74	43
Hypertension	33	19
Tendon xanthomas	3	2

CABG, coronary artery bypass grafting; CHD, coronary heart disease; MI, myocardial infarction.
Smokers currently smoked > 10 cigarettes/day or had given up less than a year before.
Hypertension was defined as a diastolic pressure > 100 mm Hg.

of any racial difference in the concentraton of serum lipids is striking since only 7% of the controls were not European, whereas 20% of the patients with coronary artery disease were immigrants, generally from the Indian subcontinent. There was a family history of coronary heart disease in 57% of the patients with coronary artery disease, of whom three (2%) had tendon xanthomas, which are indicative of familial hypercholesterolaemia.

Treatment with β blockers was an important determinant of concentrations of HDL cholesterol and apolipoprotein A-I. Mean values (SD) were lower in the 65% of patients on these drugs (0·93 (0·27) mmol/l and 123 (23) mg/dl) than in those not taking them (1·06 (0·31) mmol/l and 130 (24) mg/dl), the difference in HDL cholesterol being significant at the 1% level. The effect of these drugs on triglycerides was less pronounced, with mean values in the two groups not differing significantly (2·56 (1·44) versus 2·20 (1·21) mmol/l), and this was also true of their effect on LDL-apolipoprotein B (139 (40) versus 132 (43) mg/dl). The relative prevalence of a raised LDL-apolipoprotein B was significantly increased in normolipidaemic patients, but only if they were on β blockers (5·27 with 95% confidence limits of 1·24 to 22·47, p < 0·05).

Examination of the relation between smoking and concentrations of apolipoproteins A-I showed that mean values were lower (122 (20) mg/dl) in the 74

smokers than in the 100 non-smokers (128 (25) mg/dl), but this difference was not statistically significant. The mean value in the subgroup of 36 non-smokers not on β blockers was higher (132 (26) mg/dl) but it was still significantly lower than the mean value in controls (145 (22) mg/dl, p < 0·002). This suggests that the low concentration of apolipoprotein A-I in patients with coronary artery disease was accentuated by, but not wholly attributable to, smoking and β blockade.

DISCRIMINANT FUNCTION ANALYSIS
Stepwise discriminant function analysis was performed, after log transformation in the case of triglycerides, as follows: (a) all lipid variables in all patients and controls; (b) all lipid variables and LDL-apolipoprotein B in all patients and the apolipoprotein B subgroup of controls; (c) all lipid variables and apolipoprotein A-I in all patients and the apolipoprotein A-I subgroup of controls.

In addition, each of the three analyses was performed separately on patients grouped according to whether they were (n = 113) or were not (n = 61) receiving β blockers. The results, shown in Table 6, indicate that triglyceride was the best discriminant between patients and controls, irrespective of β blockers, and that of the lipid variables HDL cholesterol was the next best discriminator. LDL-apolipoprotein B displaced HDL-cholesterol to third place whereas apolipoprotein A-I eliminated it altogether in analyses in which each apolipoprotein was evaluated separately against the lipid variables. However, the design of the study did not permit a comparison of LDL-apolipoprotein B versus apolipoprotein A-I. Total cholesterol was an independent variable only in patients not on β blockers.

Discussion

All case-control studies are by their nature opportunistic and therefore liable to unavoidable biases, unlike

Table 6   *Variables contributing independently to the discrimination between patients and controls ranked in order of importance (p < 0·001 in every instance)*

Analysis	All subjects	No β blockers	β blockers
All lipids	(a) TG (b) HDL-C	(a) TG (b) HDL-C (c) TC	(a) TG (b) HDL-C
All lipids plus LDL-apo B	(a) TG (b) LDL-apo B (c) HDL-C	(a) TG (b) LDL-apo B	(a) TG (b) LDL-apo B (c) HDL-C
All lipids plus apo A-I	(a) TG (b) apo A-I	(a) TG (b) apo A-I	(a) TG (b) apo A-I

TG, triglyceride; TC, total cholesterol.
See text and footnote to table 1 for other abbreviations.

prospective surveys. Although this study is no exception it does give an impression of the pattern of risk factors present in men with newly diagnosed, symptomatic coronary disease in a routine clinical setting. Of the 174 patients with angiographically confirmed coronary artery disease just under 60% (104) had had a previous myocardial infarct. None had yet undergone any intervention other than treatment with antianginal drugs but 27% (47) were about to undergo coronary artery bypass grafting. Almost two thirds of the patients were on β blockers. The controls, who were drawn from men who had attended the BUPA Medical Centre for health screening during the previous three years, were free from symptoms or electrocardiographic evidence of coronary heart disease. Analysis of this group provided the cut off values used to define hyperlipidaemia and abnormally low concentrations of HDL cholesterol in patients with coronary artery disease and provided reference values for apolipoprotein A-I and LDL-apolipoprotein B. Despite possible differences in social class, occupation, and dietary habits the distribution of serum cholesterol concentrations in men attending the BUPA Medical Centre in London is known to be similar to that of men screened during the British Regional Heart Study,[16] who were chosen as being representative of the population at large.

Serum concentrations of cholesterol and triglyceride in fasting patients and controls were measured in different laboratories by similar enzymatic methods. Two methods of precipitation were used to assay HDL cholesterol but the dextran sulphate-magnesium method used to analyse samples from controls gives results that are almost identical with the modified heparin-manganese method used in the patients.[17] Interlaboratory differences were minimised by applying appropriate correction factors. Apolipoprotein A-I and LDL-apolipoprotein B were always measured by the same operators, but on separate control groups. The validity of the radial immunodiffusion method used to assay LDL-apolipoprotein B was confirmed recently[18] and the immunoturbidometric method of measuring apolipoprotein A-I gives similar results to radial immunodiffusion[19] (E Bevan and I Trayner, unpublished).

Concentrations of serum cholesterol or triglyceride (or both) were increased in 52% of the patients with coronary artery disease and 29% of the controls. In the minority of Asian patients concentrations of serum lipids were similar to those in European patients. The finding that hyperlipidaemia was twice as common in patients with coronary artery disease accords with earlier studies,[20–22] and re-emphasises the importance of blood lipids in promoting atherosclerosis. The strength of the association

between serum cholesterol and coronary heart disease mortality was underlined recently by the results of a very large prospective study,[23] which lend support to the earlier recommendations of the Consensus Development Conference on Lowering Blood Cholesterol to Prevent Heart Disease.[24] This defined 6·7 mmol/l as the concentration of total cholesterol above which there is a high risk of coronary disease in those aged 40 and over. We and the BUPA Medical Centre used this value rather than the higher 95th percentile value to define hypercholesterolaemia.

The role of triglycerides is more controversial[25] but in the present study multivariate analysis showed that hypertriglyceridaemia was the most powerful discriminant between controls and patients. This finding accords with the results of the Stockholm prospective study[26] and with Castelli's recent reappraisal of data from the Framingham Study.[27]

Hypoalphalipoproteinaemia (HDL cholesterol <5th percentile) was also more common in the patients than in the controls (31% versus 5% respectively), and the odds ratio (six) was identical to that reported by Ordovas et al.[28] Several prospective studies have found HDL cholesterol to be correlated independently (and negatively) with coronary heart disease,[29 30] but the British Regional Heart Survey was an exception because HDL cholesterol was not predictive on multivariate analysis.[31 32]

The relative prevalence of abnormally high serum concentrations of apolipoprotein B in patients not taking β blockers who had normal LDL cholesterol concentrations was 5·38 (95% confidence interval 0·72–40·19) in those with raised serum triglycerides compared with 1·03 (95% confidence interval 0·24–4·38) for those with normal triglycerides. Despite the wide confidence interval for the former group, which reflects the small numbers studied, these findings provide additional evidence of a relation between hypertriglyceridaemia and abnormally high serum concentrations of apolipoprotein B.[33] Raised concentrations of apolipoprotein B have also been reported to correlate with a family history of premature myocardial infarction[34] and have been seen in the children of such individuals.[35] This suggests a genetic basis. The increased frequency of raised concentrations of apolipoprotein B in normolipidaemic patients on β blockers, which we saw, suggests that this syndrome can also be iatrogenic.

The main new finding in this study was the presence of abnormally low serum concentrations of apolipoprotein A-I (apolipoprotein A-I value < 5th percentile despite a normal serum concentration of HDL cholesterol) in almost one third of the normolipidaemic patients with coronary artery disease. Reduced plasma concentrations of apolipoprotein A-I have been noted in the first degree relatives of patients with coronary heart disease[36 37] including their children,[38] implying that this syndrome too has

a genetic basis. In addition, environmental factors can influence plasma concentrations of apolipoprotein A-I; physical inactivity,[39] smoking,[40] and β blockers[41] all tend to reduce them. In the present study plasma concentrations of apolipoprotein A-I remained significantly lower in patients with coronary artery disease than in the controls, even when the influence of smoking and β blockers was taken into account.

Except for one small prospective study of apolipoprotein A-I[42] it remains to be established whether measurement of apolipoproteins can help to predict the risk of coronary heart disease. Analysis of restriction fragment length polymorphism of apolipoprotein genes may, however, help to define the usefulness of serum apoprotein assays as indices of genetically determined coronary disease, especially in normolipidaemic individuals.

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