Serum Lipoprotein(a) in Relation to Ischemic Heart Disease and Associated Risk Factors

by

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ABSTRACT

Serum lipoprotein(a) in relation to ischemic heart disease and associated risk factors
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Lipoprotein(a) (Lp(a)) consists of an LDL-like particle and the specific protein apo(a), which is very similar to plasminogen. Apo(a) contains repeated kringle structures and a serine protease domain, which cannot be activated by t-PA. Lp(a) is considered to be a predictor for atherosclerotic disease. It has been found incorporated in atherosclerotic plaques and inhibits in vitro fibrinolysis.

Lp(a) was determined in 1527 randomly selected individuals participating in the Northern Sweden WHO-MONICA project. A weak but significant relation between Lp(a) and increasing age was found. Menopausal status was the strongest independent predictor of Lp(a) level in women. Fibrinogen was independently related to Lp(a) in both sexes. Only a minor fraction of Lp(a) variance could be explained for in a multiple regression model, which is in agreement with the contention that Lp(a) is highly genetically determined.

Lp(a) was determined in 1571 patients investigated with coronary angiography because of suspected severe coronary artery disease (CAD). Patients with proven CAD at elective angiography had significantly higher Lp(a) than patients without significant CAD or healthy controls. Lp(a) was found to be an independent discriminator of CAD in both sexes.

HLA-DR genotype 13 or 17 was found more frequently in 30 male patients with angiographic CAD at young age (< 50 years) than in 30 age matched controls. These genotypes were common in patients with high Lp(a) levels, which indicates that Lp(a) may be related to immunological processes.

The reaction of Lp(a) was investigated in 32 patients with acute myocardial infarction (AMI). Lp(a) increased during the first week, but the response was comparatively weak. Individual Lp(a) responses were heterogeneous and no correlations to infarct size or changes in the acute phase proteins were found.

In a randomized cross-over study on 36 hypercholesterolaemic patients treated with simvastatin/placebo during 12+12 weeks Lp(a) did not change significantly, but patients with high Lp(a) levels at baseline tended to develop further increased Lp(a).

To conclude, Lp(a) was found to be an independent predictor of angiographic CAD in both men and women. Lp(a) levels are primarily genetically determined and only a small fraction of Lp(a) variance could be explained by other factors in this study. Lp(a) may be related to HLA DR types and immunological processes involved in atherosclerotic disease. Lp(a) increased slightly during the first week of AMI, but was not related to changes in the acute-phase proteins. The effective LDL-lowering agent simvastatin did not influence Lp(a) significantly.

KEY WORDS: Lipoprotein(a), lipids, epidemiology, coronary artery disease, coronary angiography, HLA DR, acute-phase protein, myocardial infarction, HMG CoA reductase inhibitor, simvastatin
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<td>acute myocardial infarction</td>
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<td>BMI</td>
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<td>CAD</td>
<td>coronary artery disease</td>
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<td>CRP</td>
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<td>DBP</td>
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<td>HDL</td>
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<td>hormonal replacement therapy</td>
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<td>IDDM</td>
<td>insulin-dependent diabetes mellitus</td>
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<td>LDL</td>
<td>low density lipoprotein</td>
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<td>Lp(a)</td>
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<td>NIDDM</td>
<td>non-insulin-dependent diabetes mellitus</td>
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<td>NSAID</td>
<td>nonsteroidal anti-inflammatory drugs</td>
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<td>PAI</td>
<td>plasminogen activator inhibitor</td>
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<td>SBP</td>
<td>systolic blood pressure</td>
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<td>SR</td>
<td>sedimentation rate</td>
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<td>TG</td>
<td>triglycerides</td>
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<td>tPA</td>
<td>tissue plasminogen activator</td>
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ABSTRACT

Lipoprotein(a) (Lp(a)) consists of an LDL-like particle and the specific protein apo(a), which is very similar to plasminogen. Apo(a) contains repeated kringle structures and a serine protease domain, which cannot be activated by t-PA. Lp(a) is considered to be a predictor for atherosclerotic disease. It has been found incorporated in atherosclerotic plaques and inhibits in vitro fibrinolysis.

Lp(a) was determined in 1527 randomly selected individuals participating in the Northern Sweden WHO-MONICA project. A weak but significant relation between Lp(a) and increasing age was found. Menopausal status was the strongest independent predictor of Lp(a) level in women. Fibrinogen was independently related to Lp(a) in both sexes. Only a minor fraction of Lp(a) variance could be explained for in a multiple regression model, which is in agreement with the contention that Lp(a) is highly genetically determined.

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HLA-DR genotype 13 or 17 was found more frequently in 30 male patients with angiographic CAD at young age (< 50 years) than in 30 age matched controls. These genotypes were common in patients with high Lp(a) levels, which indicates that Lp(a) may be related to immunological processes.

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To conclude, Lp(a) was found to be an independent predictor of angiographic CAD in both men and women. Lp(a) levels are primarily genetically determined and only a small fraction of Lp(a) variance could be explained by other factors in this study. Lp(a) may be related to HLA DR types and immunological processes involved in atherosclerotic disease. Lp(a) increased slightly during the first week of AMI, but was not related to changes in the acute-phase proteins. The effective LDL-lowering agent simvastatin did not influence Lp(a) significantly.

KEY WORDS: Lipoprotein(a), lipids, epidemiology, coronary artery disease, coronary angiography, HLA DR, acute-phase protein, myocardial infarction, HMG CoA reductase inhibitor, simvastatin
This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


II. Slunga L, Johnson O, Wester PO, Dahlén GH. Lipoprotein(a) in patients investigated with coronary angiography because of suspected severe coronary artery disease. Submitted for publication.


INTRODUCTION

Atherosclerotic disease is the predominant cause of morbidity and mortality in industrialized countries. It usually starts early in life as a silent process, which involves accumulation in the arterial intima of increasing amounts of lipid and connective tissue matrix proteins and formation of plaques. Clinical symptoms; angina pectoris, AMI or stroke, most often appear decades later and are related to plaques complications with thrombosis. The etiology of the atherosclerotic process is complex and multifactorial with both genetic and environmental, life-style factors contributing and co-operating. Hypertension, smoking, diets rich in saturated fat and cholesterol, and diabetes are some factors known to increase the risk of atherosclerotic disease. A propensity to develop early manifest atherosclerotic disease is most often related to strong, genetic factors (1, 2). Familial hypercholesterolaemia, which is characterized by defective LDL-receptors, is one example of a genetic disturbance strongly associated with early atherosclerosis. It is well-known that individuals from the general population respond very differently in lipid levels to dietary modifications. Genes probably contribute to determining the limits within which life-style factors can cause risk factor changes in a certain individual (3).

Kåre Berg reported in 1963 a new antigen in blood, the Lp(a) antigen, which was present in approximately 35% of healthy people and clearly under genetic control (4).

In 1971 Gösta Dahlén reported on a new lipoprotein fraction, which did not fit into the Fredricksson's classification of hyperlipemia (5). It was designated pre-beta1-lipoprotein due to its electrophoretic mobility. This lipoprotein fraction was detected in approximately 20% of the reference population. A highly significant association between the occurrence of this lipoprotein and clinical angina pectoris was found. In collaborative studies Kåre Berg and Gösta Dahlén established that Lp(a) lipoprotein and pre-beta1-lipoprotein were related, probably identical (6, 7).

Lp(a) lipoprotein/pre-beta1-lipoprotein was originally considered to be a qualitative trait under strong genetic control and related to atherosclerotic disease (8, 9). When sensitive immunologic testing methods became available it was made clear that Lp(a) was present in virtually all individuals and constituted a continuous variable. In further case-control studies Lp(a) lipoprotein was found to be an independent predictor for myocardial infarction (10) and angiographic CAD (11). An Lp(a) level greater than 300 mg/l was
reported to impart an increased risk for CAD (11, 12). Lp(a) has also been shown to be a significant predictor for stroke (13-15) and peripheral artery disease (16, 17). In two prospective studies Lp(a) constituted a significant predictor of CAD (18, 19). In a nested case-control study of participants in the primary preventive Helsinki Heart Study Lp(a) was, however, not found to predict future coronary events (20).

Lp(a) consists of an LDL-like particle and the specific protein apo(a), which is linked to apoB100 by a disulphide bridge (21) (Figure 1).

In 1987 McLean et al. revealed by sequencing of cloned human apo(a)-cDNA, that apo(a) was very similar to plasminogen, a plasma protein involved in the fibrinolytic process (22). Apo(a) contained a serine protease domain and two types of plasminogen-like kringle domains; a single kringle 5 and a sequence of 37 repeated kringle 4 (Figure 2). Kringles are triple-loop domains, which are common in proteins involved in the fibrinolytic system, clotting cascade and complement system. Apo(a) can not be activated by tPA or streptokinase due to a substitution of arginine to serine at the protease activation site.
Figure 2. Comparison of the gene structures of plasminogen and apo(a). S = signal peptide, T = "tail" region, K1-5 = kringle domains, P = protease domain.

Apo(a) is a large glycoprotein with a molecular weight ranging from approximately 350 to 900 kD (23). The size heterogeneity of apo(a) is due to a highly varied polypeptide chain size and a highly glycosylated structure. The interindividual variation in Lp(a) concentration is enormous. Serum Lp(a) concentration thus ranges from undetectable to more than 1000 mg/l. The distribution of Lp(a) in Caucasians is extremely skewed with most of subjects having serum Lp(a) concentrations at the low end of the range. In Africans the Lp(a) distribution is closer to normal, and the average Lp(a) level is considerably higher than in Caucasians (24, 25). A quantitative polymorphism at the apo(a) locus explains the highly varied polypeptide chain size and the large variance of the serum Lp(a) concentration (26, 27). An inverse relationship between the number of repeated kringle 4 and the serum level of Lp(a) has been found (28, 29). Different numbers of apo(a) isoforms have been detected depending on the sensitivity of the phenotyping methods used. With a high-resolution SDS-agarose electrophoresis method followed by immunoblotting 23 different apo(a) isoforms were identified (30). According to Cohen et al. it can be estimated from pulsed-field data combined with data from single-strand DNA conformation polymorphisms, that there may be more than 100 different alleles at the apo(a) locus (31). The apo(a) gene resides on chromosome six adjacent to the plasminogen gene (32). A close linkage between apo(a) isoforms and DNA polymorphisms at the plasminogen locus has been found (33).

The interest in Lp(a) has been extensive since the discovery of the structure of apo(a). Lp(a) may constitute a link between lipoproteins and thrombosis, probably having both atherogenic and thrombogenic properties. Apo(a) has been found incorporated in large amounts in arterial plaques (34) and in vein grafts (35). Kringle 4, found in plasminogen and apo(a), has lysine-binding capacity and the potential of binding to fibrinogen, fibrin products and cell
receptors (36). In vitro studies have shown that Lp(a) competes with and inhibits binding of plasminogen to fibrin products (37-39) and to cell receptors (40-42). Lp(a) also interferes with streptokinase-mediated activation of plasminogen (43, 44) and the tPA-induced lysis of fibrin clots (38).

Apo(a) is primarily synthesized in the liver (45), but mRNA for apo(a) has also been found in testis and brain tissue (46). Lp(a) is probably synthesized independently of other apoB-containing lipoproteins (47, 48). The serum level of Lp(a) is determined by the rate of synthesis (49). The further metabolism and degradation of Lp(a) is unclear. Apo(a) fragments have been detected in urine, but no relation between serum creatinine or creatinine clearance and serum Lp(a) was found (50). Conflicting results have been obtained concerning the possible catabolism of Lp(a) via the LDL-receptor. Most studies have reported a considerably lower binding of Lp(a) to the LDL-receptor as compared with LDL (51-54). However, in transgenic mice an overexpression of human LDL-receptors lead to an accelerated catabolism of Lp(a) (55).

The physiological roles of Lp(a) are obscure. Apart from in humans, Lp(a) has been detected in monkeys, principally Old World monkeys (56, 57), and in the hedgehog (58). It has been proposed that Lp(a) may be of importance for cell repair and wound healing (59).

The intraindividual level of Lp(a) has been claimed to remain very stable throughout life. Different disease states, however, affect Lp(a) levels. Increased Lp(a) levels have thus been reported in patients with end-stage renal disease or heavy proteinuria (60-63), in diabetics with nephropathy (64, 65), in patients with rheumatoid arthritis (66) and in cancer patients (67). Lp(a) levels are reduced in patients with liver cirrhosis (68, 69).
AIMS OF THE STUDIES

- To assess the distribution and variation of Lp(a) in the general population and to relate Lp(a) to other predictors for CAD (I).

- To assess the distribution and variation of Lp(a) in a population investigated with coronary angiography because of suspected severe CAD and the relation of Lp(a) to other predictors for CAD (II).

- To assess the value of Lp(a) as a predictor for CAD (II).

- To investigate whether any relations can be found between Lp(a) and HLA-DR antigens and susceptibility to early CAD (III).

- To evaluate if Lp(a) can be regarded as an acute-phase protein in acute myocardial infarction (IV).

- To investigate if treatment with the HMG CoA reductase inhibitor simvastatin influences Lp(a) level (V).
SUBJECTS, STUDY DESIGN AND METHODS

Paper I

A screening of cardiovascular risk factors in the general population in northern Sweden was performed in January to April 1990 as a part of the WHO MONICA Study. Originally 2000 individuals, 25-64-years old, stratified according to sex and age and randomly chosen from continuously updated population registers in the counties Norrbotten and Västerbotten, were invited by letter to participate in the study. In total, 1583 subjects (79.2%) accepted. Individuals who did not participate were contacted by telephone and interviewed concerning reasons for not attending, social background and cardiovascular risk factors. Results of Lp(a) analyses were available in 1527 participating subjects (76.4% of the originally invited subjects), who were subsequently enrolled in the present study.

Participants were asked to complete at home a questionnaire concerning cardiovascular risk factors, medical history and intake of drugs. Angina pectoris was diagnosed according to criteria established by Rose (70). Previous myocardial infarction was diagnosed in individuals who stated previous treatment at hospital because of definite myocardial infarction.

Two mobile teams performed the screening procedure in the whole study area. Examinations and sampling were performed between 7 a.m. and 3 p.m. Subjects who were examined before noon were instructed to be fasting since 12 hours. Data concerning last intake of food or drink were collected. It was established that 56% of the study population was at the time of sampling fasting since 12 hours. TG and calculated LDL values are only reported from fasting individuals. Total cholesterol, HDL, fibrinogen and Lp(a) are reported from all subjects irrespective of fasting time.

For calculation of the waist-to-hip ratio the smallest circumference of the waist and the thickest part of the hip in the standing position were measured. Blood pressure was measured in the sitting position after 5 min rest by a mercury sphygmomanometer using the random zero method (71). The mean of two repeated measurements was registered. Subjects were classified as hypertensive if they were treated with drugs because of hypertension or if SBP ≥160 mm Hg or if DBP ≥90 mm Hg.

Fasting subjects were examined with an oral glucose tolerance test in accordance with WHO guidelines; 75 g of glucose in 300 ml of water was
ingested over 5 min. Venous plasma samples for determination of glucose were taken immediately before the test and after 2 hours. Subjects were classified as diabetics if they already had a diagnosis of diabetes or if fasting or 2-hour plasma glucose equaled or exceeded 7.8 or 11.1 mmol/l, respectively, at an oral glucose tolerance test. An impaired glucose tolerance was diagnosed when fasting plasma glucose was below 7.8 mmol/l and 2-hour plasma glucose in the interval 7.8-11.1 mmol/l.

Paper II

From November 1987 to August 1990 a total of 1951 patients were examined with coronary angiography at the University Hospital in Umeå, because of suspected severe CAD which probably required intervention with coronary bypass grafting or angioplasty. The main indication for investigation was severe angina pectoris with restricted functional capacity. Due to insufficient data (missing records or unavailable results of Lp(a) analysis) 143 patients (7.3%) were excluded. Patients on lipid-lowering drugs ($n = 146$) and patients previously treated with coronary bypass grafting ($n = 80$) or coronary angioplasty ($n = 7$) were also excluded. The final study population consisted of 1571 patients, 1216 men and 355 women. This population was further divided into three groups; 1/ stable patients investigated with elective angiography, who had at least one significant coronary artery stenosis, 2/ unstable patients investigated with acute angiography, who had at least one significant coronary artery stenosis and 3/ patients without any significant coronary artery stenosis at angiography.

Patients were sampled, after an overnight fast, in the morning of the day of the coronary catheterization for determination of lipids and Lp(a). Samples for determination of fibrinogen and sedimentation rate were taken in a non-fasting condition on the day preceding the angiography. Clinical data were collected retrospectively from the patients medical records.

Coronary angiography was performed using Judkins' technique with multiple projections of the coronary arteries. A coronary stenosis was considered to be significant if the cross-sectional area was reduced with at least 75%. Myocardial coronary obstruction scores were calculated according to a method described by Brandt et al. (72). It implies an evaluation of the myocardial mass distal of the vessel obstruction in addition to the degree of
coronary obstruction. Collateral scores were determined by a similar method. Left ventricular motion scores were assessed by a method described by Austen et al. (73).

Cardiovascually healthy subjects from the Northern Sweden MONICA Study (579 men and 525 women) were used as referents. Subjects with a history of previous myocardial infarction or stroke were excluded as referents, as well as subjects on lipid-lowering drugs and individuals with diabetes or angina pectoris.

Paper III

Men younger than 50 and women younger than 60 years at the time of angiographically confirmed CAD, who lived in the central areas of Umeå or Skellefteå municipals, were considered for participation in the study. From January 1987 to August 1990 in total 84 patients investigated with coronary angiography fulfilled these inclusion criteria. They all had at least one significant coronary stenosis at angiography. Coronary angiography was performed according to methods described in paper II. The present follow-up and investigation with standardized sampling was performed in early 1991. At that time six of the original patients were deceased and six patients were lost. Seven patients were not included because of some recent complicating disease or cardiovascular event and five patients declined to participate. In total 60 patients (71.4% of the original population), 30 men and 30 women, agreed to participate and were included in the study. From the municipal census list of Umeå 60 sex and age matched referents were recruited. Subjects with known cardiovascular disease or diabetes were excluded.

Patients and controls were invited to the hospital in the morning, in a fasting condition since 12 hours, for sampling. Information on clinical data, heredity, menopausal status and present medication was collected. Venous blood was drawn for analysis of Lp(a) and HLA-DR genotypes.

Paper IV

Thirty-two patients (19 male and 13 female) treated at the coronary care unit at the University Hospital in Umeå because of definite AMI during late 1987 and
early 1988 were included in the study. Myocardial infarction was diagnosed by observing clinical symptoms, ECG findings and serial total creatine kinase as well as CKMB activities.

Patients treated with thrombolysis were not considered for participation because of possible effects on Lp(a) level by the thrombolytic agents. During the study period only 18% of all patients treated at the coronary care unit because of myocardial infarction received thrombolytic therapy. The main reasons for not administering thrombolytic agents were a symptom duration exceeding 4 hours, indecisive diagnosis at admission or some contraindication to thrombolysis.

Venous blood for determination of Lp(a) and the acute-phase proteins haptoglobin, orosomucoid and α1-antitrypsin was drawn in study patients at the time of admission to the coronary care unit and in the morning, after fasting overnight, on the following 6 days. The last 21 patients included in the study were also sampled daily for analysis of lipids and plasma albumin.

**Paper V**

Subjects were recruited to the study from patients who attended the Outpatients' Clinic at the Medical Department of the University Hospital in Umeå because of hyperlipidemia during the period November 1988 to February 1990. Thirty-six patients (29 male and 7 female) with total cholesterol above 6.5 mmol/l and serum Lp(a) above 100 mg/l finally participated in and completed the trial according to the protocol. Patients with a recent cardiovascular event, unstable angina, diabetes type 1, elevated liver enzymes or a secondary hypercholesterolaemia were not considered for participation as well as premenopausal women. All study patients had at baseline fasting serum TG below 4 mmol/l. All lipid-lowering drugs were stopped at least 6 weeks before the start of the baseline period and patients were instructed to follow a diet comparable to the American Heart Association Recommended Diet (phase 1) (74) during the whole study period.

The study was double-blind, placebo controlled with cross-over design. The baseline period lasted 6 weeks. Patients were then randomly allocated to receive simvastatin/placebo during a 12 week period. The daily dose of simvastatin/placebo was 10 mg during week 1-4, 20 mg during week 5-8 and
40 mg during week 9-12. After the first 12-week period each patient switched to the alternative treatment not yet received.

Patients visited the Outpatients' Clinic in the morning, having fasted for at least 12 h, in weeks -6, -2, 0 (baseline) and after 4, 8, 12, 16, 20 and 24 weeks of treatment. Determinations of total cholesterol, TG and safety laboratory tests were carried out at each visit. In weeks -6, -2, 0, 12 and 24 an extended sampling was performed, which included Lp(a), HDL, apolipoprotein A-I and apolipoprotein B. On these occasions patients also had an ECG and a physical examination was performed.

Laboratory procedures

Lp(a) lipoprotein was determined by an enzyme linked immunosorbent assay (ELISA). The detection limit was 10 mg/l and the assay range was 10 - 600 mg/l. The day-to-day CV, coefficient of variation, was 5.4%.

Total cholesterol and TG were determined by enzymatic method kits (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany). HDL-cholesterol was measured after precipitation of the other lipoproteins with sodium phosphowolframate/magnesium chloride. LDL-cholesterol was calculated by Friedewald's equation (75). Only TG values less than 4.5 mmol/l were used in the calculations. Lp(a)-adjusted total cholesterol and LDL-cholesterol were calculated with subtraction of the cholesterol present in Lp(a), (Lp(a) (mg/l)*0.3/386.7). Apoprotein A-I and apoprotein B were measured by radioimmunoassay research kits (RIA 100, Pharmacia Diagnostics, Uppsala, Sweden).

Fibrinogen was in paper I determined by the reagent kit Fibrinogen Kinetics from Boehringer Mannheim, using a Hitachi 717 analyser. Fibrinogen was in paper II determined with a thrombin reaction-rate method (BioMerieux, France). Haptoglobin, orosomucoid and α1-antitrypsin were quantified by electroimmunoassay.

CKMB activities were determined by immuno-inhibition, based on the presence of CKM-subunit antibodies.

DNA was isolated from frozen EDTA-blood according to the SDS-urea method by Lindblom and Holmlund (76). Three micrograms of DNA were cleaved with nine units of the restriction enzyme Taq I. The fragments were separated by electrophoresis in 20 cm long 0.7% agarose gels for 22 h at 40V in
Trisborate-EDTA buffer. A 1 Kb DNA ladder (BRL) was present in four lanes on each gel. The DNA was transferred to Zeta Probe membranes (BioRad) by vacuum blotting and hybridized with 32-P labelled probes. The HLA-DR types were identified by two cDNA probes; a DRB probe consisting of a 598 base pair (bp) HIND III/SacI fragment of the cDNA clone pII-β-1 (77) and a DQB probe consisting of a 627 bp AVAI fragment of the cDNA clone pII-β-1 (78).

Statistical analyses

Variables were tested for normality. The distribution of Lp(a) was highly skewed which necessitated either use of non-parametric tests or transformation to a normal distribution. Logarithms of Lp(a) were approximately normally distributed and hence used in parametric significance testing. TG and fibrinogen were also transformed to logarithms to produce normal distributions.

Independent t-tests (IV) or the Mann-Whitney U test (I, III, IV) was used for comparison of continuous variables between groups. Analysis of variance was used when adjustment for age was required (I, II). Paired t-tests were used to compare repeated determinations of variables in the same individuals (IV, V).

Pearson's correlation coefficients were calculated for normally distributed variables (IV, V). Multiple linear regression was used to study relations between variables (I, II, IV, V). A stepwise multiple linear regression model was used to assess the independent ability of different variables to predict Lp(a) level (I, II) and coronary obstruction score (II).

A discriminant analysis was carried out to assess the independent ability of lipid variables and BMI to predict patient or referent status (II).

Odds ratios for different strata of patients and referents were calculated with corresponding 95% confidence intervals (II).

The Chi-Square test with Yates' correction was used for comparing proportions (II, III).

Box-plots were constructed to illustrate serial distributions of Lp(a) (79) (IV).

A two-tailed \( p \)-value of \(< 0.05\) was chosen as the level of statistical significance.

Statistical calculations were performed in a computerized statistical program, SYSTAT\textsuperscript{®} version 5.
Ethical aspects

Informed consent was obtained from all patients and referents who entered the studies. All study protocols were approved by the Human Ethics Committee of the Medical Faculty of Umeå University.

RESULTS

Paper I

Lp(a) level did not differ between subjects who had fasted for 12 hours and those who had not. The distribution of Lp(a) was as expected highly skewed and ranged from <10 mg/l to 1291 mg/l (Figure 3). Median Lp(a) in 745 men was 103 mg/l and in 782 women 109 mg/l. An Lp(a) level exceeding 300 mg/l was detected in 18.4% of men and in 21.7% of women, and an Lp(a) level exceeding 480 mg/l in 7.8% of men and in 9.8% of women. No significant difference in Lp(a) between the sexes was found. A positive relation between Lp(a) and age was found in both men and women. Lp(a) increased almost continuously by increasing age in men, whereas in women a more distinct increase was observed in the oldest age group, 55-64 years (Table 1, I). Adjustment for different age was systematically performed in further analyses. Sexes were usually analysed separately. When numbers were small both sexes were analysed in common.

In men, an inverse relation between Lp(a) and waist-to-hip ratio was detected (standardized regression coefficient, $\beta = -0.086$, $p = 0.028$), and in women, a positive relation between Lp(a) and fibrinogen ($\beta = 0.096$, $p = 0.011$). Total cholesterol and LDL-cholesterol were significantly related to Lp(a) in both sexes, these relations however disappeared after adjustment for the content of cholesterol in Lp(a). Female smokers had significantly higher Lp(a) than non-smokers (median Lp(a) 130 and 100 mg/l, respectively, $p = 0.017$).

Individuals with a first-grade relative deceased before the age of 65 because of AMI had significantly higher Lp(a) than the rest of the study population (median Lp(a) 132 and 101 mg/l, respectively, $p < 0.05$). There was no difference in Lp(a) between individuals with a similar heredity for early death because of stroke and not. In the whole study population 29 individuals (1.9%) had clinical symptoms typical of angina pectoris, 21 individuals (1.4%) had a
history of previous AMI and 16 individuals (1.0%) a history of previous stroke. These groups, analysed separately or in common, did not have significantly different Lp(a) as compared with the rest of the population.

![Graph showing distribution of Lp(a) levels.](image)

**Figure 3.** Distribution of Lp(a) in 1527 individuals participating in the Northern Sweden MONICA Study.

A trend for lower Lp(a) in 45 diabetics as compared with 766 non-diabetics, who had been evaluated with an oral glucose tolerance test, was observed (median Lp(a) 73 and 106 mg/l, respectively, \( p = 0.051 \)). The majority of diabetics had NIDDM (29/45, 64.4%). Lp(a) levels were similar in individuals with an impaired as compared with a normal glucose tolerance.

Lp(a) was non-significantly increased in hypertensive as compared with normotensive subjects when both sexes were analysed together (median Lp(a) 115 and 102 mg/l, respectively, \( p = 0.051 \)). Significance was not obtained when sexes were analysed separately and no significant relations between SBP or DBP and Lp(a) were found.

Of the whole study population 557/1527 (36.5%) were on treatment with some kind of drug (vitamin and mineral supplements and sex hormones included). The most common drugs were aspirin, other analgesics or NSAID, beta blockers and diuretics. Subjects on diuretics (\( n = 50 \)) had significantly
higher Lp(a) than the rest of the study population (median Lp(a) 176 and 103 mg/l, respectively, \( p = 0.005 \)). In a subgroup analysis on hypertensives only, individuals on diuretics \((n = 43)\) had significantly higher Lp(a) than those on other antihypertensive agents \((p = 0.023)\). In a stepwise multiple linear regression analysis with Lp(a) as the dependent variable use of diuretics was, however, only of borderline significance \((p = 0.093)\), whereas sex and SBP were significant predictors (Table 4, I). As previously stated high Lp(a) levels were particularly common in the oldest age group of women. Of all hypertensives treated with diuretics 23/43 (53.5%) were postmenopausal women.

Healthy individuals \((n = 948)\), defined as subjects without any kind of medication, without diabetes, angina pectoris, previous AMI or stroke, had the same Lp(a) level as the rest of the population. In this subgroup of healthy individuals a significant positive relation between Lp(a) and age remained in both sexes. Cardiovascularly healthy individuals \((n = 1297)\), defined as subjects without treatment with cardiovascular drugs including antihypertensive agents, without diabetes, angina pectoris, previous AMI or stroke, had median Lp(a) 102 mg/l as compared with 125 mg/l in the rest of the population \((p = 0.090)\).

In a stepwise multiple linear regression analysis on men significant and independent predictors of Lp(a) were waist-to-hip ratio, age and fibrinogen (Table 5, I). In women menopausal status, smoking and fibrinogen were independent predictors, whereas use of diuretics was of borderline significance (Table 6, I).

**Paper II**

Both male and female patients with significant CAD at elective coronary angiography had significantly higher Lp(a) than patients without any significant stenosis. Patients with documented CAD who were investigated with acute angiography had higher Lp(a) levels than those investigated electively (Table 1, 2).
Table 1. Lp(a) levels in 1216 male patients, 31 - 80 years old, investigated with coronary angiography

<table>
<thead>
<tr>
<th></th>
<th>Significant CAD at elective angiography</th>
<th>Significant CAD at acute angiography</th>
<th>No significant CAD at angiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>996 (81.9%)</td>
<td>170 (14.0%)</td>
<td>50 (4.1%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>59.2 ± 8.1</td>
<td>60.3 ± 8.5</td>
<td>54.3 ± 9.5 ***</td>
</tr>
<tr>
<td>Lp(a) (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>238 ± 250</td>
<td>334 ± 302 ***</td>
<td>155 ± 172 *</td>
</tr>
<tr>
<td>median</td>
<td>142</td>
<td>220</td>
<td>106</td>
</tr>
<tr>
<td>Percent of patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with Lp(a) &gt; 300 mg/l</td>
<td>28.7</td>
<td>44.7 ***</td>
<td>16.0</td>
</tr>
<tr>
<td>Percent of patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with Lp(a) &gt; 480 mg/l</td>
<td>15.9</td>
<td>29.4 ***</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* p < 0.05, *** p < 0.001
Comparisons were made between patients with CAD at acute angiography or patients with no significant CAD and patients with documented CAD at elective angiography.
Table 2. Lp(a) levels in 355 female patients, 23 - 77 years old, investigated with coronary angiography

<table>
<thead>
<tr>
<th></th>
<th>Significant CAD at elective angiography</th>
<th>Significant CAD at acute angiography</th>
<th>No significant CAD at angiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>222 (62.5%)</td>
<td>63 (17.8%)</td>
<td>70 (19.7%)</td>
</tr>
<tr>
<td>Age</td>
<td>60.0 ± 8.0</td>
<td>61.5 ± 8.4</td>
<td>52.2 ± 9.8 ***</td>
</tr>
<tr>
<td>Lp(a) (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>309 ± 316</td>
<td>354 ± 304</td>
<td>186 ± 228 *</td>
</tr>
<tr>
<td>median</td>
<td>192</td>
<td>236</td>
<td>90</td>
</tr>
<tr>
<td>Percent of patients with Lp(a) &gt; 300 mg/l</td>
<td>36.0</td>
<td>42.9</td>
<td>20.0 *</td>
</tr>
<tr>
<td>Percent of patients with Lp(a) &gt; 480 mg/l</td>
<td>24.3</td>
<td>30.2</td>
<td>11.4 *</td>
</tr>
</tbody>
</table>

* p < 0.05, *** p < 0.001

Comparisons were made between patients with CAD at acute angiography or patients with no significant CAD and patients with documented CAD at elective angiography.
The further reported analyses relate only to patients with significant CAD at elective angiography. Female patients had significantly higher Lp(a) than male patients ($p = 0.004$), but in patients below 55 years of age a trend for higher Lp(a) in men was observed (Table 3, II). Lp(a) showed a weak, but significant, decreasing trend with increasing age in male patients ($p = 0.046$), whereas in women a non-significant increasing trend with increasing age was found ($p = 0.083$).

In male patients Lp(a) was inversely related to BMI and TG and positively related to HDL and SR. In female patients Lp(a) was only inversely related to TG. All relations were rather modest with standardized regression coefficients below 0.16 (Table 4, II). No relations between Lp(a) and angiographic scores were found in either sex. Male patients who were current smokers had slightly lower Lp(a) than non-smokers. Lp(a) level was considerably higher in male patients with peripheral artery disease than in patients without. A similar trend was seen in female patients. Treatment with different kind of drugs did not seem to influence Lp(a) level (Table 5, II).

A stepwise multiple linear regression analysis on male patients showed that significant independent predictors of Lp(a) level were concomitant peripheral artery disease, HDL, age, current smoking, sedimentation rate and BMI in descending order. The model was highly significant, but explained only 5.8% of the variance of Lp(a) (Table 6, II). In female patients the only significant predictor of Lp(a) level was presence of diabetes. Current smoking and peripheral artery disease were predictors of borderline significance. Age was the strongest independent predictor of the myocardial coronary obstruction score in both sexes (Table 7, II).

Cardiovascularly healthy individuals from the Northern Sweden MONICA Study were used as referents in this study. Hypertensive, but otherwise healthy, individuals were also included as referents. Since the referents all were below 65 years of age, only patients below that age were included in the further analyses. Patients with documented CAD had in both sexes significantly higher Lp(a) than the referents (Table 3, 4). When different age groups were analysed separately a significant difference in Lp(a) was only obtained in men below 55 years of age, but the proportion of subjects with an Lp(a) level exceeding 480 mg/l was increased also in patients over 55 years. A statistically significant difference in Lp(a) was obtained only in women older than 54 years, not in the younger women.
Odds ratios were calculated to study the effect of different Lp(a) levels on the risk for CAD (Table 10, II). In men below 55 years of age a significant increased risk by each Lp(a) interval was observed. In men 55-64 years old, and in women an increased risk for CAD was only seen when Lp(a) exceeded 480 mg/l.

In a discriminant analysis Lp(a) emerged as a significant, independent predictor of CAD in both men and women (Table 11, II). The strongest predictors were TG and HDL-cholesterol.

Table 3. Lp(a) levels in male patients with documented CAD at elective angiography and in referents from the Northern Sweden MONICA Study

<table>
<thead>
<tr>
<th></th>
<th>32-64 years</th>
<th>&lt; 55 years</th>
<th>&gt; 55 years</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Controls</td>
<td>Patients</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>718</td>
<td>579</td>
<td>270</td>
</tr>
<tr>
<td>Lp(a) (mg/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td>240</td>
<td>172 ***</td>
<td>261</td>
</tr>
<tr>
<td>median</td>
<td>141</td>
<td>105</td>
<td>170</td>
</tr>
<tr>
<td>Percent of patients with Lp(a) &gt; 300 mg/l</td>
<td>29.2</td>
<td>18.8 ***</td>
<td>33.0</td>
</tr>
<tr>
<td>Percent of patients with Lp(a) &gt; 480 mg/l</td>
<td>16.4</td>
<td>7.8 ***</td>
<td>18.1</td>
</tr>
</tbody>
</table>

** p < 0.01, *** p < 0.001
Table 4. Lp(a) levels in female patients with documented CAD at elective angiography and in referents from the Northern Sweden MONICA Study

<table>
<thead>
<tr>
<th></th>
<th>38-64 years</th>
<th>&lt; 55 years</th>
<th>&gt; 55 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>153</td>
<td>55</td>
<td>98</td>
</tr>
<tr>
<td>Controls</td>
<td>525</td>
<td>334</td>
<td>191</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th></th>
<th>38-64 years</th>
<th>&lt; 55 years</th>
<th>&gt; 55 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>153</td>
<td>55</td>
<td>98</td>
</tr>
<tr>
<td>Controls</td>
<td>525</td>
<td>334</td>
<td>191</td>
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<tr>
<th></th>
<th>38-64 years</th>
<th>&lt; 55 years</th>
<th>&gt; 55 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>153</td>
<td>55</td>
<td>98</td>
</tr>
<tr>
<td>Controls</td>
<td>525</td>
<td>334</td>
<td>191</td>
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<table>
<thead>
<tr>
<th></th>
<th>38-64 years</th>
<th>&lt; 55 years</th>
<th>&gt; 55 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>153</td>
<td>55</td>
<td>98</td>
</tr>
<tr>
<td>Controls</td>
<td>525</td>
<td>334</td>
<td>191</td>
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</table>

<table>
<thead>
<tr>
<th>Lp(a) (mg/l)</th>
<th>38-64 years</th>
<th>&lt; 55 years</th>
<th>&gt; 55 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>298</td>
<td>202 *</td>
<td>253</td>
</tr>
<tr>
<td>median</td>
<td>172</td>
<td>116</td>
<td>145</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Percent of patients with Lp(a) &gt; 300 mg/l</th>
<th>38-64 years</th>
<th>&lt; 55 years</th>
<th>&gt; 55 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>33.3</td>
<td>23.2 *</td>
<td>29.1</td>
</tr>
<tr>
<td>Controls</td>
<td>23.1</td>
<td>12.2 **</td>
<td>16.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Percent of patients with Lp(a) &gt; 480 mg/l</th>
<th>38-64 years</th>
<th>&lt; 55 years</th>
<th>&gt; 55 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>23.5</td>
<td>12.2 **</td>
<td>16.4</td>
</tr>
<tr>
<td>Controls</td>
<td>23.1</td>
<td>12.2 **</td>
<td>16.4</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01
Characteristics of 30 male and 30 female patients with early CAD participating in this study are given in Table 1 (III). Male patients were as selected significantly younger than female patients. Due to technical reasons results of HLA-DR typing was missing in one male and two female patients and in four controls. HLA-DR frequencies in patients and controls are given in Table 4 (III). HLA-DR 15 specificity was less frequent in male patients than in male controls \( (p = 0.039) \). The most frequent genotypes in male patients were 4, 13a and 17, but in female patients and controls of both sexes they were 1, 4 and 15. HLA-DR 13 or 17 was significantly more frequent in male patients than in male controls \( (p = 0.012) \) and HLA-DR 1, 4 or 15 less frequent \( (p = 0.051) \). In female patients a slight trend towards an increase in HLA-DR 13 was found, but the frequencies of HLA-DR 17 were similar in patients and controls. Four male patients were diabetics. A separate analysis performed with diabetics excluded showed that HLA-DR type 13 or 17 still was significantly more frequent in male patients than in controls \( (p = 0.018) \). HLA-DR 13 or 17 was common in male patients with high or medium Lp(a) levels (Table 5, III). Median Lp(a) in 15 male patients with HLA-DR 13 or 17 was 241 mg/l and in 14 male patients with other genotypes 166 mg/l \( (p = 0.167) \).

A significant positive relation was found between the relative values of Lp(a) and the time in days after the AMI event \( (p = 0.001) \). The increase in Lp(a) during the first week after AMI was, however, weak as compared with the classical acute-phase proteins; haptoglobin, orosomucoid and \( \alpha_1 \)-antitrypsin (Figure 2, IV). The individual changes in the acute-phase proteins were highly correlated to each other and to the extent of myocardial damage estimated as maximum values of CKMB. In contrast, Lp(a) did not correlate to changes in the acute-phase proteins or the CKMB values (Table III, in paper IV). The individual Lp(a) reactions were very heterogeneous. A linear regression model was used for each patient to characterize the Lp(a) reaction. An increasing Lp(a) by time was found in 10/32 (31.2%) patients, a decreasing Lp(a) in 4/32 (12.5%) and an indecisive reaction in 18/32 (56.3%). Admission values of Lp(a) did not differ between patients who showed an increase in Lp(a) and those who
did not. No relations between clinical course or drug intake and the change in Lp(a) could be detected either. Four patients were diabetics, two of them reacted with a decreasing Lp(a) level. Since glycemic control and possibly also infections might influence Lp(a) level (82), a separate analysis was performed with diabetics and patients with acute infections excluded. In this group consisting of 25 AMI patients no correlations could still be found between change in Lp(a) and change in the acute-phase proteins, and the increase in Lp(a) was still modest. A subset of 21 patients from the original study population was sampled daily for lipids and plasma albumin. Mean total cholesterol decreased by 12.5% and mean HDL-cholesterol by 21.1% from admission to day 6 in these patients. The change in Lp(a) correlated significantly with change in total cholesterol and Lp(a)-adjusted LDL-cholesterol (Table V, in paper IV). Plasma albumin decreased only slightly and non-significantly during the infarct course. A positive correlation between change in Lp(a) and change in plasma albumin was, however, found.

Paper V

Characteristics of study patients are given in Table 1 (V). Ischemic heart disease had been diagnosed in 19/36 (52.8%) and hypertension in 10/36 (27.8%) of the study subjects. Median Lp(a) at baseline was 447 mg/l. Median Lp(a) during treatment with placebo was 359 mg/l and during treatment with simvastatin 464 mg/l (p = 0.103). Two outliers in respect of Lp(a) reaction were detected (Figure 1, V). They had very high baseline Lp(a) values, 1370 and 1470 mg/ml, respectively, and both reacted with a strong increase in Lp(a) during active treatment. To weaken the effect of these outliers the values of Lp(a) change were log transformed prior to analysis with an approximate normal distribution afterwards. In a multiple linear regression analysis, change in Lp(a) was significantly related to the baseline value of Lp(a), the baseline value of apoB, and the change in TG (Table 4, V). Patients with high baseline Lp(a) were inclined to develop further increased Lp(a).
DISCUSSION

Lp(a) in the general population

Previous population studies have shown that the distribution of Lp(a) in whites and Orientals is highly skewed, whereas in blacks it is more bell-shaped and normal (24, 81, 82). Considerable differences between ethnic groups in Lp(a) levels and also in apo(a) phenotypes have been described in several studies (25, 83-86). We found in a randomly selected sample from the general population in northern Sweden, as expected, a highly positively skewed Lp(a) distribution. Comparisons of absolute Lp(a) levels obtained in different population studies are difficult since methods of Lp(a) analyses vary, and standardization of assays between different laboratories still is lacking.

We observed no significant difference in Lp(a) level between the sexes, which is in accordance with some previous reports (87, 88). However, we observed in the youngest (25-34 years) and in the oldest (55-64 years) age group a trend for higher Lp(a) in women than in men. In the Bogalusa Heart Study on children 8-17 years old a slightly higher Lp(a) level was detected in girls (82). Several authors have reported on increased Lp(a) levels in women after menopause (88, 89). In the recently presented ARIC Study, which comprises a total of 14 524 subjects of both sexes, 45-64 years old, Lp(a) was found to be slightly higher in women than in men (90).

We observed an increasing Lp(a) level by increasing age in both men and women. In women a distinct increase in Lp(a) in the oldest age group, 55-64 years, was detected, whereas in men a more continuously increasing Lp(a) level by increasing age was found. Sundell et al. also found a positive relation between Lp(a) and age in a north Swedish population consisting of 260 individuals 30-60 years old (91). They did not analyse the sexes separately. Rhoads et al. found an increased frequency of a "sinking" pre-beta lipoprotein band with increasing age in 1854 men of Japanese ancestry living in Honolulu (92). In the Framingham Offspring Study a trend for an increase in plasma Lp(a) between the ages of 20 and 59 years was observed (93), and also in the ARIC Study increasing age was associated with higher Lp(a) levels in both sexes (90). In two population studies on employees no relation could, however, be found between Lp(a) and age in men (88, 89). The relation we found between Lp(a) and age was modest. The lack of an association in some studies (88, 89, 94) may be explained by small sample sizes. Differences in selection of
study populations should also be considered. An acute-phase property of Lp(a) has been proposed (95). An increased frequency of diseases with an acute-phase reaction in elderly people could possibly explain the relation we found between Lp(a) and age. However, when we analysed only healthy individuals a significant relation between Lp(a) and age remained in both sexes.

Menopausal status was the most important independent predictor of Lp(a) level in women. Lp(a) is probably related to sex hormones in both men and women (96-99). In the present study 12.8% of postmenopausal women were on hormone replacement therapy. A trend for lower Lp(a) in these women as compared with postmenopausal women not on HRT was observed. Dahlén found decreasing Lp(a) levels in postmenopausal women, who treated with HRT (100). In the ARIC Study use of HRT was associated with a significantly decreased Lp(a) level (90). We could not detect any influence of hormonal contraceptives on Lp(a). The number of women taking hormonal contraceptives was low. Relations between Lp(a) and other hormones; thyroid hormones and growth hormone, have been reported (101-103).

We found in both sexes a weak, but independent and significant, relation between Lp(a) and fibrinogen, which is in accordance with results from the PROCAM study on employees (89). We did not analyse relations between Lp(a) and other fibrinolytic variables. Most previous studies have not detected any relations between Lp(a) and the fibrinolytic variables PAI and tPA (91, 104, 105). However, in the PROCAM study an inverse relation between Lp(a) and PAI-1 was detected in multiple regression analysis.

Lp(a) has been shown to be mainly independent of other cardiovascular risk factors. In several studies no associations between Lp(a) and diet (106, 107), weight or body-fat distribution (84, 91, 108) were observed. A declining Lp(a) level in men and women during weight reduction has been reported in one study (109), but initial Lp(a) values in that study did not correlate to overweight or body fat distribution. Some authors have, however, found discrete relations between Lp(a) and body weight (88, 89, 92). We did find in men a discrete inverse relation between Lp(a) and waist-to-hip ratio. No similar relation was observed in women. We could not detect any relation between physical activity and Lp(a). Hellsten et al. found declining Lp(a) levels in 16 well-trained men, who participated in a cross-country skiing tour in the Swedish mountains at winter time (110).

Most studies report no effect of smoking on Lp(a) (7, 84, 88, 89, 111). We observed no relation between smoking and Lp(a) in men, but in women
smoking was associated with a higher Lp(a) level. The increased Lp(a) level in female smokers may be explained by the anti-estrogen effect induced by smoking (112, 113).

Discrete correlations between SBP and Lp(a) were observed in the two previously mentioned studies on employees (88, 89). We could not detect any significant relations between blood pressure levels and Lp(a). A trend for higher Lp(a) in hypertensive as compared with non-hypertensive individuals was, however, found. Subjects on diuretics had particularly high Lp(a). The reason for increased Lp(a) in individuals on diuretics is difficult to establish. The main indication for treatment with diuretics was hypertension. More than half of treated subjects were postmenopausal women. In a multivariate analysis on hypertensives only, sex and systolic BP were significant predictors of Lp(a), whereas use of diuretics only was of borderline significance. There is very sparse information in the literature concerning the effect of different cardiovascular drugs on Lp(a). Donders et al. observed increased Lp(a) levels in 54 patients with essential hypertension and unsatisfactory blood pressure level (114).

Davies et al. found increased Lp(a) in subjects with an impaired glucose tolerance (115). We could not confirm that finding. The effect of diabetes on Lp(a) is somewhat controversial. The main evidence points towards normal Lp(a) levels in diabetics with good metabolic control and without nephropathy (116-121). We found slightly (non-significantly) lower Lp(a) in diabetics as compared with non-diabetics.

A small proportion of the general population in our study had clinical overt cardiovascular disease. These individuals did not differ significantly in Lp(a) level as compared with the rest of the study population. A strong heredity for early death because of AMI was, however, associated with a high Lp(a), which is in accordance with the concept that Lp(a) is strongly determined by genetic factors. In multiple regression analysis we also found that only very small proportions of Lp(a) variance could be explained by external or life-style factors.

Lp(a) in coronary artery disease

An increased Lp(a) level was found in patients with confirmed CAD at angiography both in comparison with patients without any significant CAD at
angiography and in comparison with healthy referents from the Northern Sweden MONICA Study. These findings are in agreement with results from most previous angiographic studies (9, 122-125). However, Nieminen et al. could not detect any difference in Lp(a) level between 111 Finnish patients with angiographic CAD and 46 patients without CAD at angiography or 96 healthy controls (126). Their study included both males and females and also patients evaluated with angiography because of valvular heart disease. A different selection of study subjects and a rather small sample size may, at least in part, explain the divergent results obtained in this study. Ethnical differences in predictors of angiographic CAD may also be of importance.

We excluded from our study patients on lipid-lowering drugs and patients who previously had been treated with coronary bypass grafting or angioplasty. This probably implies that individuals with the most pronounced lipid disturbances were left out. Treatment with lipid-lowering agents was, however, relatively uncommon during the study period. In an Italian study on 1200 hyperlipidemia patients, an increased Lp(a) level was observed in subjects on lipid-lowering drugs, especially in patients receiving multiple lipid-lowering agents (127). We did find in our original material a trend for increased Lp(a) in patients who were excluded from the study because of treatment with lipid-lowering agents. An Lp(a) level exceeding 300 mg/l was significantly more common in these patients \( p < 0.05 \) than in patients with proven CAD at elective angiography who were not on lipid-lowering drugs. HMG CoA reductase inhibitors were the most common lipid-lowering drugs used in our patients. Patients excluded from our study because of previous coronary bypass surgery did not differ in Lp(a) level as compared with included patients.

Patients who did not show any significant CAD at angiography constituted a heterogeneous group. A majority of them were females. It is well established that CAD is more difficult to establish from anamnesis and exercise test in females as compared with males. Common diagnoses in patients without significant CAD at angiography were hypertension, probable spasm angina, pains of non-coronary origin and suspected syndrome X. Some patients had discrete coronary sclerosis, but no significant stenoses.

The referents selected from the Northern Sweden MONICA Study were judged to be cardiovascularly healthy because of no history of a previous AMI or stroke, no current symptoms typical of angina pectoris and no treatment with lipid-lowering drugs. They were, however, not evaluated with either ECG,
exercise test or coronary angiography, which probably implies that some cases with asymptomatic CAD were included as referents. Asymptomatic CAD may be encountered as frequently as in 2.5 to 10 percent of middle-aged men (128, 129). We excluded diabetics as referents since an increased prevalence of asymptomatic CAD has been reported in diabetics (130-132).

Patients with confirmed CAD at acute angiography had significantly higher Lp(a) than patients going through an elective evaluation. This may be due to increasing Lp(a) levels after AMI and possibly also during unstable ischemic events (133). An increased Lp(a) level may also predispose to acute, unstable courses.

Lp(a) levels in different age groups of patients with proven CAD at elective angiography differed between male and females. High Lp(a) levels were particularly common in young male patients (< 55 years) and in older female patients (> 55 years). Dahlén et al. found significantly elevated Lp(a) only in men younger than 55 years, which is in agreement with our results (11). The incidence of CAD is low in women below the age of 55 years. It increases however rapidly thereafter. The pathogenic mechanisms of CAD in women before and after menopause may differ, probably because of alterations of sex hormones. Lp(a) is known to increase after menopause and, according to our results, seems to constitute a stronger predictor for CAD in women older than 55 years than in younger women.

We found, in agreement with previous reports (12, 124), that Lp(a) was mainly unrelated to other established predictors of CAD. Only small proportions of Lp(a) variance could be explained by other known factors in multiple regression analyses. However, in men Lp(a) was weakly, but independently, related to HDL-cholesterol and inversely to BMI. High Lp(a) levels accordingly seem to be more common in male patients without derangements typical of the metabolic syndrome. In bivariate analysis a significant inverse relation between Lp(a) and TG was detected in both sexes. In multiple regression analysis the relation, however, did not remain. This was probably due to the well-known inverse relation between HDL and TG. In the Italian study on hyperlipidaemic patients a reduced Lp(a) level was observed in patients with hypertriglyceridemia as compared with other hyperlipidemias, and an inverse relation between TG and Lp(a) was detected in both sexes (127). These results are in accordance with data from our CAD patients. We could not detect any significant relation between TG and Lp(a) in the general population. The observed inverse relation between TG and Lp(a) is probably most
prominent in populations where hypertriglyceridemia is common. The mechanisms behind the association between Lp(a) and TG are not fully known. Apo(a) has been detected in TG-rich lipoprotein fractions postprandially and after fat ingestions (134-138). The association of apo(a) with TG-rich lipoprotein particles may influence the metabolism of Lp(a) and also affect the total serum concentration. We could not confirm the finding by Armstrong et al. that an increased LDL concentration markedly increased the risk of CAD due to elevated Lp(a) (12). Current smoking was associated with a slightly lower Lp(a) value in our patients, and in female patients also diabetes was associated with a lower Lp(a). These findings probably can be explained by a considerably increased risk for CAD in smokers and in diabetics even if the Lp(a) level is low or moderate. Werba et al. found in hyperlipidemic subjects significantly lower Lp(a) in smokers than in non-smokers (127). Winocour et al. have reported on lower Lp(a) in diabetic versus non-diabetic individuals with clinically diagnosed CAD (139).

Although Lp(a) levels are generally considered to be very stable through life, a reactive increase in Lp(a) may occur in diseases with an inflammatory component (66, 95). In analogy to these findings Lp(a) could be reactively elevated also in atherosclerotic disease. Sandholzer et al. have shown that apo(a) isoforms differ between CAD patients and controls in several different ethnic populations (140). In a study on individuals with a heredity for NIDDM, similar Lp(a) levels were found in diabetics with previous AMI and in their healthy relatives (121). These findings support the contention that Lp(a) is an inherited factor related to CAD. Even if Lp(a) is to some extent reactively increased in CAD patients it may nevertheless exert a harmful effect promoting atherosclerosis.

We found in elective male patients with established CAD a weak, but significant and independent, relation between Lp(a) and SR. Lp(a) is a huge lipoprotein particle, which could possibly in high concentrations lead to an increased SR. We could not detect any relations between Lp(a) and SR or fibrinogen in unstable male CAD patients, but in female CAD patients investigated acutely a comparatively strong relation between fibrinogen and Lp(a) was observed.

Lp(a) was not related to the angiographic scores. We used a coronary obstruction score which takes into consideration both the degree of obstruction and the myocardial mass distal of the stenosis. The majority of our CAD patients had an extensive coronary sclerosis. Attempts to further grade the
disease in this highly selected population may be rather meaningless. We included in the analysis only individuals with significant CAD. In some previous studies both patients with and without significant CAD at angiography were included in the analysis. Age was the strongest predictor of the coronary obstruction score in both sexes in our study. Hearn et al. found similar Lp(a) levels in subjects with different numbers of diseased coronary vessels (123).

A discriminant analysis was carried out to assess the independent ability of lipid variables to discriminate between CAD patients and healthy controls. Adjustment for the effect of some possible confounders was performed simultaneously. Lp(a) constituted an independent discriminator of CAD in both sexes of approximately the same strength as Lp(a)-adjusted LDL-cholesterol. The most potent discriminators were, however, TG and HDL-cholesterol. These results are in accordance with several previous reports (11, 123, 124, 141, 142).

Pathogenic mechanisms in Lp(a) related atherosclerotic disease

The structure of Lp(a) suggests that it has both atherogenic and thrombogenic properties. The exact pathogenic mechanisms in Lp(a)-associated atherosclerotic disease are, however, not yet fully elucidated.

Several mechanisms through which Lp(a) may inhibit fibrinolysis have been described. In vitro studies have shown that Lp(a) competes with plasminogen for binding to endothelial cells and thereby inhibits fibrinolysis on cell surfaces (40-42). Lp(a) also inhibits activation of plasminogen by streptokinase (44) or tPA, and it competes with plasminogen for binding to fibrin (37-39). A relation between Lp(a) and the fibrinolytic capacity in vivo has been much more difficult to assess (105, 125, 143), and Lp(a) does not seem to affect the outcome of thrombolytic therapy (144, 145).

Cushing et al. detected high concentrations of apo(a) in resected vein grafts from patients undergoing coronary re-bypass surgery, but virtually no apo(a) in normal saphenous veins (35). Apo(a) is probably selectively retained in vein grafts since the apo(a)/apoB ratio was higher in graft tissue than in plasma. Beisiegel et al. performed biochemical and immunohistochemical studies of biopsies from fresh human arterial walls (146). They found an accumulation of intact apo(a) in the arterial intima, preferentially extracellularly in plaque areas,
where it was strongly co-localized with apoB and fibrin. In some cases they also detected apo(a) in foam cells. Krempler et al. showed that Lp(a)-dextran sulphate complexes were incorporated in the same extent as modified LDL in mouse peritoneal macrophages (147). The uptake of Lp(a) into the arterial intima is probably mediated by its strong binding to glycosaminoglycans (148, 149). Dahlén et al. showed in an early in vitro study that binding of Lp(a) to glycosaminoglycans was minimal at physiological ionic strength, but increased considerably and exceeded that of LDL after addition of calcium ions (148). Lp(a) is the only lipoprotein which precipitates at physiological ionic strength and calcium ion concentration, a property which should facilitate uptake in macrophages (150-152). Lp(a) has furthermore been shown to bind to fibronectin (153), fibrin (38) and plasminogen receptors on endothelial cells (42). Lp(a) could thereby inhibit fibrinolysis at the endothelial surface, and both fibrin and Lp(a) would be internalised into the arterial intima.

Components of the immune system are known to be involved in the atherosclerotic process (154). Activated T-lymphocytes, macrophages and immunoglobulins have been detected in large numbers in atherosclerotic plaques (155, 156). Several cytokines are also produced in atherosclerotic lesions (157). The scavenger receptor, which is responsible for cholesterol uptake in macrophages, is under cytokine control (158). Oxidized LDL particles and oxidation products are known to be strong antigens and may elicit production of autoantibodies (159). The protein part of lipoproteins may also be processed in macrophages and thereafter presented to T-cells in combination with HLA-DR antigens on the macrophage surface, which could lead to T-cell activation. Lp(a) has a strong tendency to aggregate, it can also form aggregates with LDL, preferably modified LDL (160). These aggregates could be taken up in macrophages and constitute important initiators of the immunological process in the arterial intima.

We postulated a hypothesis that certain HLA class II genotypes in conjunction with Lp(a) could influence T-cell activation and thus initiate or modulate the immune response present in atherosclerosis. The atherosclerotic process itself could also possibly stimulate the synthesis of Lp(a). We found in a pilot study on 30 men with angiographic CAD before the age of 50 an increased frequency of HLA-DR 13 or 17 as compared with 30 age matched healthy controls. A trend for higher Lp(a) values in patients with these genotypes as compared with other genotypes was found. The results of this pilot study should be interpreted with some caution, but if confirmed by larger studies
they could help to explain why some patients with high Lp(a) and cholesterol levels develop early atherosclerosis while others with comparable lipid levels do not. An over-representation of HLA-DR 13 or 17 could not be found in female patients with early CAD. The pathogenic mechanisms in early CAD in women may, at least partly, differ from those in men.

Individuals with the inherited disease homocystinuria are characterized by very high plasma concentrations of the amino acid homocysteine and a strongly increased risk to develop cardiovascular disease at young age. Some recent studies have shown that individuals with a moderate hyperhomocysteinemia also carry an increased risk to encounter cardiovascular disease (161). An interesting relation between Lp(a) and plasma homocysteine has been described by Harpel et al. (162). They found that homocysteine, as well as other sulphydryl-containing amino acids, enhanced the binding of Lp(a) to fibrin. This effect on Lp(a) could at least partly explain the atherogenic properties of homocysteine.

Lp(a) in the acute phase reaction

Plasma proteins which show fast and reversible changes in concentration during conditions characterized by cell injury are called acute-phase reactants. An acute-phase reaction may be encountered both in acute and chronic diseases. Important acute-phase proteins are CRP, α₁-antitrypsin, orosomucoid, haptoglobin and fibrinogen. They are all synthesised in the liver. Different cytokines probably mediate the increased synthesis of these proteins in conditions with cell injury. Several of the acute-phase proteins contain large amounts of sialic acid. Lp(a) is also enriched in sialic acid and has been proposed to constitute an acute-phase protein in inflammatory conditions. Rantapää et al. observed increased Lp(a) levels in patients with rheumatoid arthritis (66). Maeda et al. have previously reported on increasing Lp(a) levels during the course of AMI and after different kinds of surgical operations (95).

We investigated Lp(a) levels and the acute-phase proteins orosomucoid, α₁-antitrypsin and haptoglobin in 32 patients treated because of AMI. We excluded patients treated with thrombolysis since the thrombolytic agents could possibly influence Lp(a). We found, in accordance with Maeda et al., an increase in the relative Lp(a) levels, but the increase in Lp(a) was weak as compared with increases in the classical acute-phase proteins. The change in
Lp(a) varied considerably between different patients. We could not detect any correlations between the change in Lp(a) and the changes in the acute-phase proteins or the extent of myocardial damage estimated as the maximum CKMB value. In 21 patients we also analysed lipids daily and in this subset of patients we found a significant correlation between the individual change in LDL-cholesterol (adjusted for Lp(a)) and the change in Lp(a). Inflammatory conditions are known to be accompanied by decreases in HDL- and LDL-cholesterol (163). The mechanisms behind these transient effects on lipoproteins are not fully settled. Alterations in the synthesis or catabolism of lipoproteins or an altered intravascular metabolism are some suggested mechanisms. A changed distribution of HDL-lipoprotein particles between the intra- and extravascular space has also been suggested. Johansson et al. distinguished three different patterns of response in plasma proteins during AMI (164). CRP, haptoglobin, fibrinogen, orosomucoid and α1-antitrypsin showed a rapid increase within some days, whereas ceruloplasmin and C3 showed a moderate increase with a maximum level during the second week. Albumin, transferrin and immunoglobulin G reacted with a rapid decrease the first week. We followed Lp(a) during six days after the admission to the coronary care unit. The peak in Lp(a) level was seen at day 5. According to other studies Lp(a) rises relatively slowly and reaches a peak 2 weeks after AMI (95, 165). The lack of correlation between change in Lp(a) and change in classical acute-phase proteins during the first week after AMI was anyway unexpected. Mechanisms other than the acute-phase reaction also influencing Lp(a) should be considered. Plasma albumin is known to decrease during AMI, probably because of a decreased synthesis in the liver and a shift to the extracellular space. We analysed plasma albumin in the subset of 21 patients. We only detected a weak, non-significant, decline in albumin during the infarct course, but we found a significant correlation between the individual change in albumin and the change in Lp(a). Change in albumin also correlated inversely to CKMB; patients with high CKMB tended to have larger declines in albumin. Linear regression analysis was therefore performed with Lp(a) as the dependent variable and with concomitant adjustment for albumin levels. Lp(a) did show a slightly stronger increase by time in this analysis, but still no positive relations between the change in Lp(a) and the change in the acute-phase proteins could be found. The clinical course in AMI is of course very varied. Patients with small infarctions may develop unstable conditions afterwards and patients with large infarctions are inclined to develop heart
failure. Complications like infections are not uncommon. Glycemic control and fluid balance may also change. Patients are usually treated with many different drugs which might influence Lp(a). We could not detect any influence of some clinical variables on the Lp(a) reaction, but the numbers were of course small. Oshima et al. found increasing Lp(a) in patients treated because of unstable angina (133). Acute ischemic events without cell injury may accordingly influence Lp(a). To conclude, we found that Lp(a) increased moderately during the first week after myocardial infarction, but it did not react in accordance with the classical acute-phase proteins. The Lp(a) reaction appeared instead to be related to the change in LDL-cholesterol and the change in plasma albumin.

Treatment of elevated Lp(a) levels

Different diets used to lower LDL-cholesterol do not influence Lp(a) levels significantly (106, 107). A diet high in trans-mono unsaturated fatty acids has been reported to increase Lp(a) (166). The majority of lipid-lowering drugs used today do not seem to affect Lp(a). The bile acid sequestrants do not have any effect on Lp(a) (167, 168). Most studies report no major effect of fibrates on Lp(a) (169-171). Maggi et al., however, observed reduced Lp(a) levels in 21 patients treated with bezafibrate during six months (172). Nicotinic acid alone or in combination with neomycin reduces Lp(a) by 38-45% (173, 174). Treatment with nicotinic acid is, however, restricted because of troublesome adverse reactions.

The HMG CoA reductase inhibitors are potent LDL-lowering agents. They increase the clearance of LDL into the hepatocytes via the LDL-receptor. We investigated the effect of simvastatin on Lp(a) in 36 hypercholesterolemic patients in a randomized, placebo controlled cross-over study. A slight increase in Lp(a) was found during active treatment as compared with placebo, but the difference was not significant. Patients with high baseline Lp(a) values had a tendency to react with a further increase in Lp(a). Increased Lp(a) levels during treatment with reductase inhibitors have been reported previously (175, 176). The main evidence, however, points towards no major effects of the reductase inhibitors on Lp(a) (177-179). This finding indicates that catabolism via the LDL-receptor does not determine Lp(a) level to any major degree.

Probucol does not affect Lp(a) levels, but it protects Lp(a) against oxidative modification and inhibits further uptake in macrophages (180, 181).
Antioxidants may, according to these results, be promising agents for treatment of subjects with high Lp(a) values. Fish oil with high levels of ω3 fatty acids do not seem to exert any major effects on Lp(a) (182-184). N-acetylcysteine is a reducing agent which like homocysteine has the potential of cleaving disulphide bonds. It was first reported that n-acetylcysteine had a profound decreasing effect on serum Lp(a) (185). In later experiments Scanu et al. found that n-acetylcysteine and other cysteine-containing compounds attenuated the immunoreactivity of Lp(a) (186). Harpel et al. found an increased affinity of Lp(a) to fibrin after treatment with n-acetylcysteine (162). N-acetylcysteine may thus, in a similar way as plasma homocysteine, exert an unfavourable effect on Lp(a) and promote atherosclerosis. Apheresis effectively lowers serum Lp(a) by 40-60% (187, 188). It may constitute a suitable treatment in familial hypercholesterolemia and other conditions with severe lipid disturbances.

To conclude, the possibilities to treat elevated Lp(a) levels are today rather restricted as the majority of the traditional lipid-lowering agents do not influence Lp(a). No study has either thus far addressed the potential benefit in risk for atherosclerotic disease of lowering Lp(a). The general opinion today is that other risk factors should be treated vigorously when a high Lp(a) value is detected. Use of estrogens in hormonal replacement therapy to postmenopausal women is probably advantageous.

CONCLUSIONS

Only small proportions of the variation of serum Lp(a) in the general population, as well as in patients with CAD, could be explained by external or life-style factors, which is in accordance with the concept that serum Lp(a) is strongly determined by genetic factors. Lp(a) was mainly unrelated to other established cardiovascular risk factors. We did, however, in the general population find a discrete relation between Lp(a) and fibrinogen and a slightly increasing Lp(a) level by increasing age. Menopausal status was the strongest predictor of Lp(a) in women. Women who smoked had an increased Lp(a) level, which may be related to effects on estrogens. In male patients with CAD Lp(a) was weakly related to HDL-cholesterol and inversely to BMI. Lp(a) constituted an independent discriminator of angiographic CAD in both men and women. High Lp(a) levels were particularly often found in young male patients and in elderly female patients with CAD. Lp(a) is probably a stronger
risk factor for CAD in women after menopause than before. Patients with confirmed CAD who were investigated with acute angiography had significantly higher Lp(a) than patients investigated electively.

Several plausible mechanisms to explain the atherothrombotic effect of Lp(a) have been proposed. In a pilot study we found preliminary support of a hypothesis that Lp(a) may be related to HLA-DR antigens and possibly constitutes an initiator or modulator of the immune response found in atherosclerotic processes. Lp(a) probably increases in conditions characterized by an acute-phase reaction, but we could not relate the changes in Lp(a) to changes in the classical acute-phase reactants. Lp(a) was not significantly influenced by treatment with the HMG CoA reductase inhibitor simvastatin. A trend for further increased Lp(a) in individuals with high baseline Lp(a) values was, however, found.

Both epidemiological and experimental studies thus support the concept that high Lp(a) levels predispose to atherosclerotic disease. However, to assess a confident causal relationship between Lp(a) and atherosclerosis further prospective and interventional studies are requested as well as basic research on pathogenic mechanisms. Regard should also be taken to the heterogeneity of Lp(a) lipoprotein particles both within the same individual and between individuals. The same quantitative result of immunological apo(a) testing may signify different degrees of atherogeneicity in different individuals.

A large-scale screening of Lp(a) in the general population is probably not sensible today. In patients with an established increased risk for cardiovascular disease, especially with a hereditary component, Lp(a) should be determined. The means to treat elevated Lp(a) levels are rather restricted today, but hopefully new effective, interventional therapies will be developed in Lp(a)-associated atherosclerotic disease.
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