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# Lipoprotein Lp(a) as predictor of myocardial infarction in comparison to fibrinogen, LDL cholesterol and other risk factors: results from the prospective Göttingen Risk Incidence and Prevalence Study (GRIPS)

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**Abstract.** Based on pathophysiological findings Lp(a) is considered to be a cardiovascular risk factor. The Göttingen Risk Incidence and Prevalence Study (GRIPS) provides the possibility to evaluate this impact of Lp(a) on the basis of a large prospective cohort study. GRIPS included 6002 men, aged 40–59·9 years at baseline. Data of a 5 year follow-up period is now available for >95% of the study participants. Multivariate logistic regression models for the estimation of MI risk confirm Lp(a) as an important risk factor, ranking fifth behind LDL cholesterol, family history of MI, plasma fibrinogen and HDL cholesterol (inversely related). The GRIPS data strongly support strategies for the identification and treatment of persons at increased MI risk which focus on LDL cholesterol. However, Lp(a) and fibrinogen have to be seriously considered as additional risk factors and should be included in diagnostic panels for the estimation of MI risk.

**Keywords.** Fibrinogen, LDL cholesterol, lipoprotein(a), myocardial infarction.

## Introduction

Lipoprotein (a) (Lp(a)) resembles low density lipoproteins (LDL) in being rich in cholesterol and apolipoprotein B-100, but it is distinguished by its content of apolipoprotein apo(a) [1]. This apolipoprotein exhibits a genetically determined size heterogeneity which is inversely associated with the Lp(a) plasma concentration, accounting for up to, or even more than, 50% of its variance in Western populations [2]. Apo(a) was characterized as a glycoprotein with a structural homology to plasminogen [3] causing an inhibition of plasminogen binding and activation [4,5]. Although the metabolism of Lp(a) is not yet fully elucidated, there is evidence that these particles exhibit a low affinity to the LDL-receptor and may be eliminated from the plasma by alternative, possibly atherogenic, pathways [6]. Furthermore, apo(a) was found to be present in atherosclerotic plaques [7].

Additionally, fibrinogen and its split products are contained in atherosclerotic lesions [8]. It was suggested that fibrinogen and its degradation products contribute to atherogenesis by several mechanisms: increasing the permeability of endothelium for plasma proteins; providing an adsorptive surface for LDL accumulation; and stimulating smooth muscle cell proliferation and migration [8].

Based on these pathophysiological findings, Lp(a) and fibrinogen were suggested to be independent risk factors for atherosclerotic disease [6,9]. As to fibrinogen, this idea is supported by prospective cohort studies [10–12]. Lp(a) was positively related to cardiovascular disease in several clinical and epidemiological studies (predominantly retrospective case control studies), although there are also contradictory results [13–19]. This is particularly true for the two available prospective case control studies. Rosengren *et al.* [19] reported increased Lp(a) baseline values in subjects with myocardial infarction during a follow-up period of 6 years as compared to randomly selected controls, whereas Jauhiainen *et al.* did not find such differences even with a very similar study design [18].

In order to evaluate the role of Lp(a) as a risk factor for atherosclerotic diseases, in particular for myocardial infarction, and to place it in a ranking with other risk factors including LDL cholesterol and fibrinogen, a large prospective cohort study is needed. The Göttingen Risk Incidence and Prevalence Study (GRIPS) is such a prospective cohort study including about 6000 male participants, aged 40–59·9 years at study entry. Results based on the first 5 year follow-up period will be presented in this paper.

The present results of the study were presented in part at the 26th Annual Scientific Meeting of the European Society of Clinical Investigation, 1992.

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## Participants and methods

Details concerning the design and organization of the Göttingen Risk Incidence and Prevalence Study have been presented elsewhere [20,21], and are therefore described only in brief.

### *General design*

GRIPS is an ongoing prospective cohort study. The baseline investigation included 6002 men, aged 40–59·9 years. According to anamnestic data and non-invasive clinical examinations 5728 of these subjects were free of atherosclerotic diseases (coronary heart disease, myocardial infarction, peripheral arterial vascular disease, stroke) at study entry. They represent the definitive study group and are prospectively observed by follow-up investigations in order to record morbidity and mortality rates. Until now 5 year follow-up data of 5471 subjects (i.e. 95·5% of the definitive study group) are available.

### *Endpoints*

Individuals who developed one of the following primary endpoints during the follow-up period are considered as incidence cases of myocardial infarction (MI) ($n=107$): definite sudden coronary death ($n=4$); definite fatal MI ($n=23$); definite non-fatal MI ($n=80$).

Individuals who developed one of the following secondary endpoints are excluded from the evaluations in this presentation: definite chronic coronary heart disease (CHD) without MI ($n=73$); definite stroke (fatal or non-fatal) ($n=49$); definite peripheral arterial vascular disease (PAVD) ($n=26$); suspect MI, CHD, stroke or PAVD ($n=14$); death from non-cardiovascular causes ($n=78$). 5124 subjects from the definite study group remained free of any of the endpoints and are thus considered as a reference group.

The definite and suspect endpoints are defined according to widely accepted recommendations of other epidemiological studies [22–24] as described in detail elsewhere [20,25]. The diagnosis of endpoints is based on clinical symptoms, resting and exercise ECG, enzyme activity pattern, angiography and computer tomography.

### *Baseline variables*

The major baseline parameters of GRIPS are as follows:

*Anamnestic variables*:

date of birth;
history of diseases and medications;
family history of myocardial infarction (0 vs. $>=1$ first-degree relative with MI before the age of 65);
smoking habits: smokers (subjects smoking daily at the time of baseline investigation) vs. non-smokers (ex-smokers were not distinguished from subjects who had never smoked since the respective information was of minor reliability);
alcohol consumption (0–1 days/week, 2–4 days/week, $>=5$ days/week in the following referred to as never, occasionally or regularly). Since the MI incidence was similar in subjects consuming alcohol occasionally or regularly these subgroups are combined for the present evaluations;
sporting leisure time activities (less than once per week vs. at least once per week in the following referred to as rarely or regularly).

*Clinical variables:*

body mass index calculated as weight (kg) height $(m)^{-2}$; blood-pressure (mean of 3 measurements by a mercury sphygmometer; systolic blood-pressure beginning of the first Korotkoff phase; diastolic blood-pressure change from third to fourth Korotkoff phase).

*Laboratory variables:*

total cholesterol, triglycerides, LDL cholesterol, VLDL cholesterol, HDL cholesterol, apoprotein B, apoprotein AI, Lp(a), fibrinogen, plasma glucose, uric acid.

### *Laboratory methods*

Total serum cholesterol and triglycerides were measured 2 years after the baseline investigation in sera stored at −90°C, by enzymatic procedures (CHOD-PAP and GPO-PAP, respectively) with reagents from Boehringer, Mannheim, Germany, using an automated analyser Hitachi 705. LDL, VLDL and HDL cholesterol were directly measured in freshly drawn sera by quantitative lipoprotein electrophoresis (qlE) [26]. These analyses were validated 6 years later by precipitation techniques for the determination of LDL cholesterol (precipitation with dextrane sulphate; Immuno GmbH, Heidelberg, Germany) [27] or HDL cholesterol (after precipitation of apo B containing lipoproteins with sodium phosphotungstate/$MgCl_2$; Boehringer, Mannheim, Germany), respectively. Results of qlE were considered as invalid if they were different from those of the respective precipitation technique by more than 10% for LDL cholesterol or more than 15% for HDL cholesterol. For such sera, definite lipoprotein concentrations were obtained by ultracentrifugation according to LRC recommendations [28]. In all other samples qlE results were used for the present report.

The apoproteins B and AI, as well as plasma fibrinogen, were measured 6 years after the baseline investigation, in samples stored at −90°C, by nephelometry using a nephelometer analyser BNA as well as monospecific antisera from Behring AG, Marburg, Germany.

Lp(a) was determined 9 years after the baseline investigation in sera stored at −90°C by the commercially distributed one-step sandwich ELISA 'Immuno-

zym Lp(a)' (Immuno GmbH, Heidelberg, Germany). The wells of the ELISA test-strips were coated with a monospecific polyclonal anti-apo(a) antibody which recognizes all apo(a) isoforms and does not cross-react with plasminogen. Diluted serum samples (1:501) were incubated for 2 h in these wells, together with a conjugated solution containing a monospecific monoclonal anti-apo(a) antibody linked to peroxidase. All further steps of the test procedure were performed exactly as prescribed by the manufacturer. The absorbance of the final enzymatic colour reaction was read at 450 nm by a Dynatech MR 5000 reader (Dynatech, Denkendorf, Germany). Lp(a) values were calculated as total Lp(a) mass from a standard curve constructed for each plate using a commercially available Lp(a) reference standard (Immuno GmbH, Heidelberg, Germany). The Lp(a) concentration of this standard material was defined by the manufacturer using a purified Lp(a) preparation as primary standard.

It is a controversial issue whether Lp(a) can be correctly measured in frozen sera. We therefore repeated the Lp(a) determination by the ELISA technique in freshly drawn sera of about 200 GRIPS participants 9 years after the baseline investigations and compared the results with those obtained in the above-mentioned frozen sera of the same individuals. The correlation coefficient of 0·93 and the regression line of $y = 0{\cdot}98x + 0{\cdot}15$ suggest a correct measurement of Lp(a) in the −90°C frozen samples.

Standard and control materials for lipid, lipoprotein, apoprotein and fibrinogen measurements were from Behring AG, Marburg, and Immuno GmbH, Heidelberg, Germany. In addition, the precision of the lipid, lipoprotein and apolipoprotein analyses was controlled by self-prepared pooled sera. During the whole period of measurements of each parameter a single lot number of the respective reagent, control and standard material was used. Coefficients of variation (within/between run) were as follows:

Total cholesterol, triglycerides 0·6/1·0–1·3%;
LDL cholesterol (qlE or precipitation) 1·3–1·5/1·7–2·0%;
HDL cholesterol (qlE or precipitation) 1·5–2·0/2·5–3·5%;
Apo AI, apo B, fibrinogen 1·6/2·5–3·0%;
Lp(a)1·5/3·5–4·0%.

*Statistical methods*

For continuous variables correlation coefficients were computed according to the methods of Pearson or, for Lp(a), Spearman, respectively. To assess the relationship between continuous and dichotomous variables the point-biserial correlation coefficient was computed [29]. These calculations were performed for the total definitive study group of 5728 subjects irrespective of their participation in the follow-up period. To test the differences of risk factors between the reference and the incidence group the two-tailed *t*-test was used for continuous variables except for Lp(a) for which the difference was tested with the Kolmogorov–Smirnov test. For dichotomous variables the $\chi^2$ test was used and relative risks are computed together with 95% confidence intervals. Relative risks for continuous variables were calculated by dividing the reference and incidence group into three subgroups according to tertiles which were derived from the distribution of values in the total definitive study group. The relative risks, together with the respective 95% confidence intervals, are calculated using the category with the lowest MI incidence as a baseline. All above-mentioned analyses were carried out on an IBM compatible PC using SAS statistical software [30].

To study the joint relationship of potential risk factors to MI risk, and to calculate adjusted odds ratios, multivariate logistic regression analyses were carried out on a Siemens P 7·580–S computer using BMDP statistical software [31]. The anamnestic variables, smoking habits, alcohol consumption, sporting activities and family history of MI entered the analyses as described in the section Baseline variables. Age, as well as all clinical and laboratory data, were also categorized for the analyses except for total cholesterol, LDL cholesterol, apo B and the ratios LDL/HDL cholesterol and apo B/apo AI, which were used as continuous variables implying a dose–response relationship to MI. LDL cholesterol additionally entered the analyses as coded into three strata ($<3{\cdot}9$, 3·9–4·9, $\geq 4{\cdot}9$ mmol $l^{-1}$; $<150$, 150–190, $\geq 190$ mg $dl^{-1}$). The monotone relationship of LDL cholesterol to MI was proven by comparing the likelihood-ratio statistics and odds ratios of a model including two dummy variables for the three categories vs. a model with one interval-scaled variable defined by equidistant codes.

Forward and backward selection was used to build up the logistic regression model. Both procedures are modified in that at each point of the selection process the partial significance of each term included in, or excluded from, the model is reviewed. In our analyses the criterion for a variable to enter and to remain in the model was that its initial probability value as well as its partial probability value in the presence of other variables should not exceed 0·01. Maximum likelihood statistics was used for the selection process. As a last step interactions between the variables remaining finally in the model were tested.

## Results

*Interdependencies between baseline variables*

In the GRIPS study group the baseline variables revealed well known and expected interdependencies, most of them being of minor relevance (Table 1). This is particularly true for Lp(a). However, some apparent relationships existed, especially of fibrinogen to age or smoking, triglycerides to body mass index and HDL cholesterol to body mass index or alcohol consumption.

**Table 1.** Correlation coefficients for selected baseline variables ($n = 5728$)

| Variable | LDL chol. | HDL chol. | Triglycerides | Lp(a) | Fibrinogen |
|---|---|---|---|---|---|
| Lp(a)* | 0·03 | 0·05 | 0·03 | 1·00 | 0·00 |
| Fibrinogen† | 0·14 | −0·06 | 0·06 | 0·00 | 1·00 |
| Plasma glucose† | 0·01 | −0·04 | 0·11 | 0·01 | 0·05 |
| Body mass index† | 0·11 | −0·21 | 0·20 | −0·04 | 0·09 |
| Age† | 0·08 | −0·05 | 0·01 | −0·01 | 0·36 |
| Blood-pressure (WHO)‡ | 0·05 | −0·04 | 0·11 | −0·02 | 0·11 |
| Smoking‡ | 0·06 | −0·07 | 0·04 | −0·01 | 0·16 |
| Alcohol‡ | −0·04 | 0·16 | 0·03 | −0·01 | −0·05 |
| Sport‡ | −0·01 | 0·05 | −0·05 | 0·01 | −0·06 |
| Family history of MI‡ | 0·06 | −0·04 | 0·05 | 0·03 | −0·02 |

*Spearman correlation coefficient; †Pearson (column 'Lp(a)' Spearman) correlation coefficient; ‡point-biserial correlation coefficient.

**Table 2.** Comparison of MI incidence and reference group for continuous variables. Data for Lp(a) are given as median and percentiles (10–90%), data for the other variables are presented as mean values (standard deviations)

| Variable | MI incidence cases Mean | Reference group Mean | *P*-value* |
|---|---|---|---|
| Cholesterol mmol $l^{-1}$ | 6·52 (1·03) | 5·59 (1·02) | <0·001 |
| Triglycerides mmol $l^{-1}$ | 2·03 (0·84) | 1·73 (0·9) | <0·001 |
| LDL chol. mmol $l^{-1}$ | 4·69 (0·89) | 3·72 (0·85) | <0·001 |
| HDL chol. mmol $l^{-1}$ | 1·11 (0·26) | 1·26 (0·31) | <0·001 |
| VLDL chol. mmol $l^{-1}$ | 0·72 (0·41) | 0·61 (0·44) | <0·01 |
| Apo B mg $dl^{-1}$ | 139·4 (24·72) | 119·2 (23·7) | <0·001 |
| Apo AI mg $dl^{-1}$ | 118·9 (23·15) | 126·1 (27·78) | <0·01 |
| LDL/HDL cholesterol | 4·46 (1·31) | 3·15 (1·11) | <0·001 |
| Apo B/Apo AI | 1·22 (0·32) | 0·99 (0·32) | <0·001 |
| Fibrinogen mg $dl^{-1}$ | 423·7 (96·97) | 363·8 (84·48) | <0·001 |
| Systolic BP mmHg | 138·2 (18·22) | 131·3 (15·33) | <0·001 |
| Diastolic BP mmHg | 89·2 (9·27) | 85·6 (8·87) | <0·001 |
| Plasma glucose mmol $l^{-1}$ | 6·15 (2·19) | 5·66 (1·48) | <0·05 |
| Uric acid mmol $l^{-1}$ | 1·02 (0·21) | 1·01 (0·21) | NS |
| Body mass index kg $m^{-2}$ | 26·70 (3·49) | 26·18 (2·96) | NS |
| Age years | 49·95 (4·92) | 47·54 (5·06) | <0·001 |
| | Median | Median | *P*-value† |
| Lp(a) mg $dl^{-1}$ | 18 (<5–63) | 9 (<5–42) | <0·001 |

BP, blood-pressure; **t*-test; †Kolmogorov–Smirnov test.

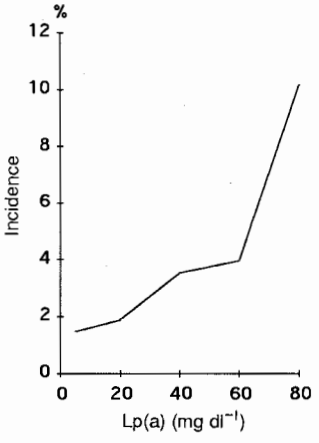


**Figure 1.** Impact of Lp(a) concentration at baseline on the MI incidence during 5 year follow-up.

*Association of MI incidence with Lp(a) and other risk factors*

The MI-incidence patients revealed markedly increased Lp(a) values as compared to the reference group (Table 2). Accordingly the MI incidence increased consistently and significantly with increasing Lp(a) values (Fig. 1).

Based on the results of the univariate statistical analyses, the strength of the association of Lp(a) with the MI incidence is comparable to that of several other risk factors such as age, HDL cholesterol, apo AI, family history of MI, triglycerides, VLDL cholesterol, blood-pressure or smoking habits, but stronger than that of plasma glucose, body mass index, uric acid, alcohol consumption or sporting activities (Tables 2, 3, 4, Fig. 2B, D, F). In contrast, according to univariate analyses, the relationships of total cholesterol, LDL cholesterol, apo B, LDL/HDL cholesterol ratio, apo

**Table 3.** Comparison of MI incidence and reference group for dichotomous variables by means of $\chi^2$ test

| | Prevalence | | | | | | |
|---|---|---|---|---|---|---|---|
| | MI cases | | Ref. group | | | | |
| Trait | *n* | % | *n* | % | RR | 95% CI | *P*-value |
| Hypertension (WHO) | 32 | 29·9 | 948 | 18·5 | 1·9 | 1·2–2·8 | <0·01 |
| Smoking (yes) | 58 | 54·2 | 1864 | 36·4 | 2·0 | 1·4–2·9 | <0·001 |
| Alcohol (never) | 24 | 22·4 | 559 | 10·9 | 2·3 | 1·5–3·6 | <0·001 |
| Sports (rarely) | 76 | 71·0 | 3101 | 60·5 | 1·6 | 1·1–2·4 | <0·05 |
| Family history of MI (yes) | 32 | 29·9 | 449 | 8·8 | 4·2 | 2·9–6·1 | <0·001 |

RR, relative risk; CI, confidence interval; *n*, number of subjects.

B/apo AI ratio or fibrinogen to the MI incidence (Tables 2, 4, Fig. 2A, C, E) are stronger than that of Lp(a).

For the majority of the tested variables the results of the univariate analyses are strongly supported by multivariate logistic regression analyses, which were performed in order to build up a model for the estimation of MI risk.

Two alternative models were finally chosen, each of them yielding identical results by forward, as well as backward, selection:

Model A with LDL cholesterol stratified as $<3{\cdot}9$, $3{\cdot}9$–$4{\cdot}9$, $>=4{\cdot}9$ mmol $l^{-1}$ ($<150$, 150–190, $>=190$ mg $dl^{-1}$), but used as an interval-scaled variable;

model B with LDL cholesterol as a continuous variable.

It is important to note that both models revealed LDL cholesterol as the strongest predictor of MI and were virtually identical concerning the ranking and the odds ratios of the other variables. As indicated by model A (Table 5) LDL cholesterol is followed by family history of MI, plasma fibrinogen, HDL cholesterol (inversely related) and Lp(a). Age was of considerably minor importance as MI predictor in the GRIPS study group.

Total cholesterol, apo B and the ratios LDL/HDL cholesterol or apo B/apo AI respectively did not enter the final prediction model if tested together with LDL cholesterol. However, each of these variables was able to replace LDL cholesterol as the strongest predictor in the model if tested instead of it. Likewise, apo AI was eliminated from the final prediction model by HDL cholesterol.

Plasma glucose, smoking and blood-pressure failed to enter the final prediction model on the basis of a significance level of $P<0{\cdot}01$, but would have been included (ranking 7th to 9th without influence on the ranks or the odds ratios of the more powerful predictors) by reducing the significance level to $P<0{\cdot}05$ or $0{\cdot}1$, respectively. Triglycerides and VLDL cholesterol totally failed to enter the final prediction model even on the basis of a significance level of $P<0{\cdot}1$.

Among the tested interactions between the variables of the final prediction models A and B only a negative, but insignificant ($P=0{\cdot}13$), interaction between Lp(a) and LDL cholesterol is noteworthy.

Specificity and sensitivity in estimating MI risk as obtained by model A (Table 5) in an internal validation are given in Fig. 3 by a ROC curve.

Based on model B, Fig. 4 shows the relationship of the 5 year MI risk to the LDL cholesterol concentration. This relationship is presented for different subgroups with various panels of additional risk factors. This figure provides the possibility to demonstrate the cumulative impact of multiple risk factors.

It becomes clearly evident that at any LDL cholesterol level other risk factors (e.g. Lp(a)) potentiate the LDL-induced MI risk. For example, at a LDL cholesterol level of 4·9 mmol $l^{-1}$ (190 mg $dl^{-1}$), which is generally considered as very high, the MI risk is clearly below the average MI incidence of the GRIPS study group (2% in 5 years), provided no additional risk factor is present. The same LDL cholesterol level induces an approximately average MI risk if it is associated with one additional risk factor, for example Lp(a) $>=30$ mg $dl^{-1}$. In combination with two additional risk factors, for example Lp(a) $>=30$ mg $dl^{-1}$ and HDL cholesterol $<0{\cdot}9$ mmol $l^{-1}$ (35 mg $dl^{-1}$), this very same LDL cholesterol value of 4·9 mmol $l^{-1}$ (190 mg $dl^{-1}$) is associated with a MI risk clearly above the 2% average.

## Discussion

Lipoprotein Lp(a) has been considered to be a risk factor for early development of atherosclerotic disease [6]. However, until now no data from a prospective cohort study were available to evaluate the impact of Lp(a) on the MI risk in comparison with other variables. The Göttingen Risk Incidence and Prevalence Study provides the basis for such an analysis.

The results presented concerning the prediction models for MI may be considerably influenced by the way the various variables are entered into the multivariate logistic regression analyses. Applying logistic regression as a first step, a decision has to be made on the categorization of continuous variables. The use of a continuous variable in a logistic regression model implies a log-linear influence of this variable on MI risk. Among the tested variables this is likely only for total cholesterol, LDL cholesterol, apo B, the ratios

**Table 4.** Relative risk of MI for continuous variables in terms of thirds

| Variable | *n* (MI) | *n* (R) | Rate/1000 | RR | 95% CI | *P*-value |
|---|---|---|---|---|---|---|
| Cholesterol mmol $l^{-1}$ | | | | | | |
| <5·1 | 9 | 1698 | 5·3 | | | |
| 5·1–6·0 | 18 | 1790 | 10·0 | 1·9 | 0·9–4·1 | NS |
| >=6·1 | 80 | 1636 | 46·6 | 8·8 | 5·0–16 | <0·001 |
| Triglycerides mmol $l^{-1}$ | | | | | | |
| <1·3 | 20 | 1733 | 11·4 | | | |
| 1·3–1·8 | 36 | 1719 | 20·5 | 1·8 | 1·1–3·1 | <0·05 |
| >=1·9 | 51 | 1672 | 29·6 | 2·6 | 1·6–4·2 | <0·001 |
| LDL chol. mmol $l^{-1}$ | | | | | | |
| <3·3 | 8 | 1751 | 4·5 | | | |
| 3·3–4·0 | 19 | 1714 | 11·0 | 2·4 | 1·1–5·3 | <0·05 |
| >=4·1 | 80 | 1659 | 46·0 | 10·1 | 5·7–18 | <0·001 |
| HDL chol. mmol $l^{-1}$ | | | | | | |
| >=1·4 | 17 | 1665 | 10·1 | | | |
| 1·1–1·3 | 28 | 1734 | 15·9 | 1·6 | 0·9–2·8 | NS |
| <1·1 | 62 | 1725 | 34·7 | 3·4 | 2·1–5·7 | <0·001 |
| VLDL chol. mmol $l^{-1}$ | | | | | | |
| <0·41 | 23 | 1824 | 12·5 | | | |
| 0·41–0·65 | 37 | 1664 | 21·8 | 1·7 | 1·1–2·9 | <0·05 |
| >=0·66 | 47 | 1636 | 27·9 | 2·2 | 1·4–3·6 | <0·01 |
| Apo B mg $dl^{-1}$ | | | | | | |
| <109 | 11 | 1696 | 6·4 | | | |
| 109–127 | 24 | 1614 | 14·7 | 2·3 | 1·1–4·5 | <0·05 |
| >=128 | 67 | 1659 | 38·8 | 6·0 | 3·5–10 | <0·001 |
| Apo AI mg $dl^{-1}$ | | | | | | |
| >=134 | 28 | 1716 | 16·1 | | | |
| 112–133 | 33 | 1661 | 19·5 | 1·2 | 0·7–2·0 | NS |
| <112 | 42 | 1593 | 25·7 | 1·6 | 1·0–2·6 | <0·05 |
| LDL/HDL cholesterol | | | | | | |
| <2·6 | 9 | 1710 | 5·2 | | | |
| 2·6–3·4 | 16 | 1776 | 8·9 | 1·7 | 0·8–3·8 | NS |
| >=3·5 | 82 | 1638 | 47·7 | 9·1 | 5·2–16 | <0·001 |
| Apo B/Apo AI | | | | | | |
| <0·85 | 13 | 1695 | 7·6 | | | |
| 0·85–1·08 | 23 | 1682 | 13·5 | 1·8 | 0·9–3·5 | NS |
| >=1·09 | 66 | 1592 | 39·8 | 5·2 | 3·1–8·8 | <0·001 |
| Lp(a) mg $dl^{-1}$ | | | | | | |
| <5 | 22 | 1652 | 13·1 | | | |
| 5–17 | 27 | 1716 | 15·5 | 1·2 | 0·7–2·1 | NS |
| >=18 | 58 | 1756 | 32·0 | 2·4 | 1·5–3·9 | <0·001 |
| Fibrinogen mg $dl^{-1}$ | | | | | | |
| <326 | 15 | 1775 | 8·4 | | | |
| 326–394 | 27 | 1721 | 15·4 | 1·8 | 1·0–3·4 | NS |
| >=395 | 65 | 1628 | 38·4 | 4·6 | 2·8–7·6 | <0·001 |
| Systolic BP mmHg | | | | | | |
| <125 | 21 | 1676 | 12·4 | | | |
| 125–139 | 37 | 1715 | 21·1 | 1·7 | 1·0–2·9 | <0·05 |
| >=140 | 49 | 1733 | 27·5 | 2·2 | 1·4–3·6 | <0·01 |
| Plasma glucose mmol $l^{-1}$ | | | | | | |
| <5·2 | 35 | 1902 | 18·1 | | | |
| 5·2–5·7 | 22 | 1614 | 13·4 | 0·7 | 0·4–1·3 | NS |
| >=5·8 | 50 | 1608 | 30·2 | 1·7 | 1·1–2·5 | <0·05 |
| Uric acid mmol $l^{-1}$ | | | | | | |
| <0·92 | 29 | 1637 | 17·4 | | | |
| 0·92–1·07 | 34 | 1674 | 19·9 | 1·1 | 0·7–1·9 | NS |
| >=1·08 | 44 | 1813 | 23·7 | 1·4 | 0·9–2·2 | NS |
| Body mass index kg $m^{-2}$ | | | | | | |
| <25 | 30 | 1729 | 17·1 | | | |
| 25–26 | 33 | 1650 | 19·6 | 1·1 | 0·7–1·9 | NS |
| >=27 | 44 | 1744 | 24·6 | 1·4 | 0·9–2·3 | NS |
| Age years | | | | | | |
| <44 | 17 | 1579 | 10·7 | | | |
| 44–49 | 31 | 1864 | 16·4 | 1·5 | 0·9–2·8 | NS |
| >=50 | 59 | 1681 | 33·9 | 3·2 | 1·9–5·3 | <0·001 |

*P*-values were calculated using the $\chi^2$ test; *n*(MI), number of incidence cases; *n*(R), number of reference subjects; RR, relative risk; CI, confidence interval; BP, blood-pressure.

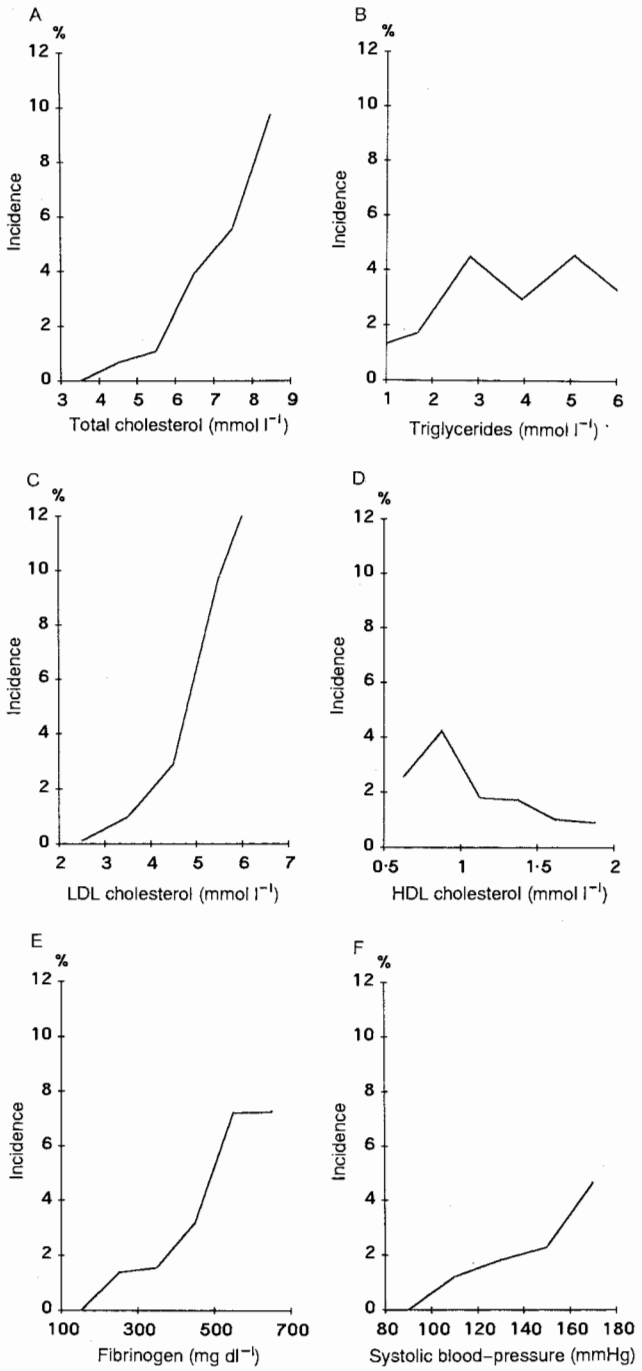


**Figure 2.** Impact of various variables at baseline on the MI incidence during 5 year follow-up. (A) Total cholesterol; (B) triglycerides; (C) LDL cholesterol; (D) HDL cholesterol; (E) fibrinogen; (F) systolic blood-pressure.

LDL/HDL cholesterol and apo B/apo AI and possibly for fibrinogen. A further reason for categorizing variables is to provide the possibility of estimating relative risks by computing odds ratios which give a very distinct measure of the impact of the respective variable on MI risk. The choice of a cut-off point has to be made primarily on medical aspects, but in addition should yield a reasonable number of subjects in each group. Widely accepted threshold values were used for triglycerides (2·3 mmol $l^{-1}$; 200 mg $dl^{-1}$), HDL cholesterol (0·9 mmol $l^{-1}$; 35 mg $dl^{-1}$), VLDL cholesterol (0·8 mmol $l^{-1}$; 30 mg $dl^{-1}$), Apo AI (110 mg $dl^{-1}$), plasma glucose (6·7 mmol $l^{-1}$; 120 mg $dl^{-1}$) and uric acid (1·2 mmol $l^{-1}$; 7·0 mg $dl^{-1}$). Two cut-off points are usually discussed for Lp(a) (20 or 30 mg $dl^{-1}$), body mass index (25 or 30 kg $m^2$) and blood-pressure (140/90 or 160/95 mmHg according to World Health Organization (WHO) criteria), respectively. Each was tested in the logistic regression model and the final choice was made on the basis of best model fit and highest likelihood ratio $\chi^2$. For fibrinogen (in particular for the immunologic measurement used in GRIPS), at the time of the evaluation of this study, no reliable recommendations existed concerning a threshold value for elevated levels, which are suspected to induce an increased risk for MI. We decided to test two cut-off points (400 and 420 mg $dl^{-1}$, respectively) which are near the 75% percentile. This seemed reasonable since the latter was also true for the above-mentioned cut-off points of the other variables, although these had been defined according to clinical aspects. We also took into account sensitivity and specificity of these points and, as already mentioned, the number of subjects falling into each category.

However, it should be pointed out that the choice of different threshold values for the various variables, or their use in a categorized instead of an interval-scaled form, did not change the results concerning LDL cholesterol. In all models tested, LDL cholesterol proved to be the most important risk factor for MI out of our comprehensive panel of variables. Thus the GRIPS data strongly support the strategies of the National Cholesterol Education Program (NCEP) [32] and more recent recommendations of our study group [20,33] for the identification of persons at increased MI risk, both of which focused on LDL cholesterol as the predominant predictor and the most important target for treatment. It is important to note that in GRIPS, LDL cholesterol was measured directly by two different methods of particularly high interassay precision (coefficient of variance CV $< = 2\%$) and not calculated by the Friedewald formula with its rather poor precision (CV $> 5\%$).

Furthermore, the GRIPS data give support to the view that the individual MI risk as derived from LDL cholesterol is strongly augmented if additional risk factors are present. In particular, our data indicate that Lp(a) has to be seriously considered if the MI risk of a subject is to be determined. Earlier findings of our group, derived from a retrospective case control study [16], suggested that Lp(a) is of particular interest if associated with borderline or elevated LDL cholesterol concentrations. The present prospective GRIPS data are in accordance with this finding. They indicate that elevated Lp(a) is of clinical importance, particularly in subjects with borderline LDL cholesterol, since their MI risk is increased from below average (in the absence of other risk factors) to average or above

**Table 5.** Ranking of risk factors for the estimation of MI risk evaluated by means of multivariate logistic regression analysis (model A)

| Variable | Categories | Coefficient | Standard error | Odds ratio | 95% CI | $\chi^2$ | *P*-value |
|---|---|---|---|---|---|---|---|
| LDL cholesterol | $<3{\cdot}9/3{\cdot}9$–$4{\cdot}9/>=4{\cdot}9$ mmol $l^{-1}$ | 1·273 | 0·140 | 3·6 | 2·7–4·7 | 86 | <0·001 |
| Family history of MI | no/yes | 1·436 | 0·237 | 4·2 | 2·6–6·7 | 31 | <0·001 |
| Fibrinogen | $<420/>=420$ mg $dl^{-1}$ | 0·994 | 0·215 | 2·7 | 1·8–4·1 | 21 | <0·001 |
| HDL cholesterol | $>=0{\cdot}9/<0{\cdot}9$ mmol $l^{-1}$ | 1·018 | 0·234 | 2·8 | 1·8–4·4 | 17 | <0·001 |
| Lp(a) | $<30/>=30$ mg $dl^{-1}$ | 0·887 | 0·217 | 2·4 | 1·6–3·7 | 16 | <0·001 |
| Age | $<50/>=50$ years | 0·603 | 0·216 | 1·8 | 1·2–2·8 | 8 | <0·01 |
| Constant | | −6·248 | 0·258 | | | | |

Odds ratio, exp (regr. coefficient); $\chi^2$ and *P* values according to likelihood ratio statistics; CI, confidence interval.

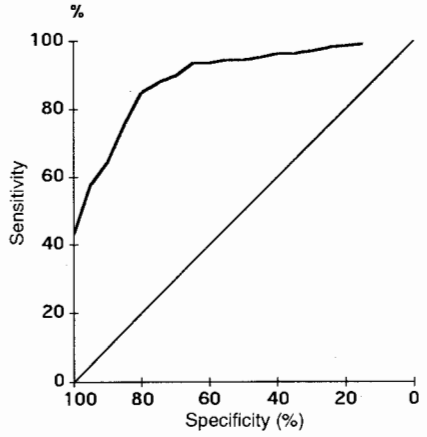


**Figure 3.** ROC curve for model A as given in Table 5.

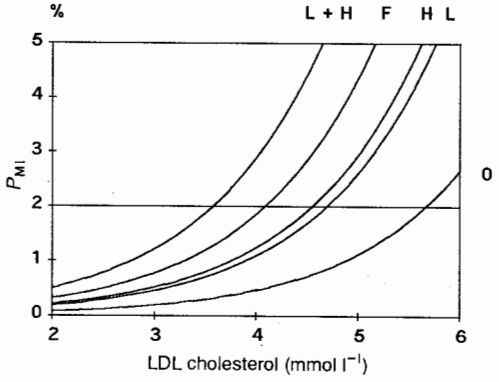


**Figure 4.** Five year probability of MI according to LDL cholesterol concentration in subgroups stratified for the presence of various risk factors (RF). O, no additional RF; L, Lp(a)>=30 mg $dl^{-1}$; H, HDL cholesterol <0·9 mmol $l^{-1}$; F, positive family history of MI.

average when Lp(a) concentrations >=30 mg $dl^{-1}$ are present. However, in the GRIPS cohort high Lp(a) concentrations are associated with an increased MI risk also in subjects with rather low (<3·9 mmol $l^{-1}$; 150 mg $dl^{-1}$) LDL cholesterol levels. In this subgroup the relative risk of elevated Lp(a) concentrations is even more striking than in those with higher LDL cholesterol concentrations, although the absolute risk remains below average. These findings thus indicate a negative interaction between LDL and Lp(a) concerning their impact on the MI risk. This is also a considerable, although insignificant, trend in the multivariate logistic regression analyses which may indicate that in this case the multiplicative model is not adequate. This aspect needs further investigation.

In the GRIPS cohort the impact of increased Lp(a) concentrations as MI risk factor was as strong as that of a positive family history of MI, hyperfibrinogenaemia or decreased HDL cholesterol. Thus, increased Lp(a) values rank among the strongest MI risk factors, but behind LDL cholesterol.

Total cholesterol, apo B and the ratios LDL/HDL cholesterol or Apo B/Apo AI did not enter the final estimation model for MI risk. This was obviously due to their strong physiologically explicable correlations with the even stronger predictor LDL cholesterol. Likewise, apo AI was excluded from the final prediction model because of its strong correlation with HDL cholesterol. Thus, it is important to note that apo B and apo AI were less effective predictors of MI than LDL or HDL cholesterol, respectively.

Triglycerides and VLDL cholesterol revealed a considerable impact on the MI risk only in the univariate analyses. Both variables did not significantly contribute to the improvement of the multivariate prediction model. This finding is in accordance with other reports [34], and may be due to the fact that the distribution of triglycerides among the various lipoprotein families is not sufficiently elucidated by the laboratory methods used.

Hypertension, hyperglycaemia and smoking failed to enter the logistic regression model using a significance level of $P<0{\cdot}01$. Their *P*-values ranged around 0·05 depending on details (e.g. threshold values and mode of selection) of the respective analysis. Thus their ranking as risk factors of MI is relatively weak in the GRIPS study group as compared to other reports [35,36]. However, in most of the respective studies important variables such as Lp(a), fibrinogen or family history of MI were only partly, or not at all, recorded.

One might suspect that in GRIPS the recording of blood-pressure and smoking habits was performed with less accuracy as compared to that of the various laboratory parameters, leading to an under-estimation of these variables in regard to their real impact on the MI risk. It could also be argued that plasma glucose

levels are insufficiently representative of glucose metabolism and diabetes mellitus. However, these three variables proved to be by far the most important risk factors for the secondary endpoints of our study, namely stroke and peripheral arterial vascular disease [37], as was to be expected with respect to other reports [38,39].

Our data on smoking support earlier findings from the Framingham study, indicating that smoking is no longer an independent risk factor if fibrinogen is incorporated in multivariate logistic regression analysis [12].

On the basis of the GRIPS data presented in this report we believe that there is a need to include Lp(a) and fibrinogen in diagnostic panels for the estimation of MI risk. They should also be considered when defining the target LDL cholesterol value in the treatment of hypercholesterolaemia and CHD.

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