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SERUM LIPOPROTEINS AND CORONARY ARTERY DISEASE (CAD)

Comparison of the Lipoprotein Profile with the Results of Coronary Angiography

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Summary

The presence or absence of coronary artery disease was established by coronary angiography in 181 male patients (aged 40—60 years). The concentrations of cholesterol and triglycerides were determined in the sera of all patients. In addition, plasma lipoproteins were quantified by a recently developed quantitative lipoprotein electrophoresis based on densitometric scanning of lipoprotein bands visualized by polyanion precipitation after electrophoretic separation. The most pronounced differences between these two groups of patients were found in the concentrations of whole serum cholesterol, β-lipoprotein cholesterol and the β-lipoprotein/α-lipoprotein ratio. No marked differences were seen in the concentrations of serum triglycerides, pre-β-lipoprotein cholesterol or α-lipoprotein cholesterol. A combination of critical values for the concentrations of serum cholesterol and β-lipoprotein cholesterol and for the β-lipoprotein/α-lipoprotein ratio could be established. If exceeding at least two of the three critical values was used as the cut-off point between the two groups of patients, a maximum differentiation of 50% could be achieved (81% correctly classified patients vs. 31% incorrectly classified).

Introduction of the β-lipoprotein/α-lipoprotein ratio as criterion shifts the range of differentiation favorably, increasing it by about 10%. This effect cannot be achieved by regarding the level of α-lipoprotein cholesterol as criterion.

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Key words: *Cholesterol — Coronary angiography — Coronary artery disease — HDL cholesterol — Serum lipoproteins*

Introduction

At the present time there is little doubt that the elevated serum cholesterol levels and the incidence rate of ischemic heart disease (IHD) are closely connected. The fact that the increased risk for IHD in hypercholesterolemic patients is due to an elevated concentration of low density lipoproteins (LDL) has been repeatedly demonstrated [1,2] and is nowadays well accepted. IHD, however, can very often be detected in patients exhibiting normal serum cholesterol values. This fact can be due either to accumulation of other risk factors, such as cigarette smoking or hypertension in those patients, or to an increase in the plasma of LDL counterbalanced by a decreased concentration of high density lipoproteins (HDL), or a combination or both. Indeed there are many reports in the literature [3–5] about the negative correlation of the HDL-concentration and the incidence of IHD. This finding has been substantiated by the detection of a cholesterol-mobilizing effect of HDL in experiments designed to study the interaction of fibroblasts or smooth muscle cells with isolated serum lipoproteins [6].

The methods used for lipoprotein quantification in epidemiologic studies have to be precise, but simple and easy to perform. These criteria are in general fulfilled by precipitation methods for HDL quantification. The concentration of LDL is usually assessed by calculation from serum triglyceride and cholesterol concentrations in combination with the HDL-cholesterol value [7], since no simple method for direct measurement of LDL cholesterol has been available. This, however, is now possible using quantitative lipoprotein electrophoresis based on densitometric scanning of the lipoprotein bands after separation by agarose gel electrophoresis and subsequent visualisation by polyanion precipitation [8].

Using this method for direct quantification of the serum lipoproteins of patients undergoing coronary angiography, we tried to arrive at an answer to the following questions concerning the predictive value for IHD of the concentrations of the different serum lipoproteins alone or in combination.

(1) Is there a certain value of LDL cholesterol which can serve as cut-off point between patients with and without angiographically proven coronary artery (CAD)?

(2) Can this cut-off point be provided by a combination of serum lipoprotein concentrations? Lipoprotein electrophoresis is especially suited to establish such combinations.

(3) Can HDL exert the protective effect over a wide range of LDL concentrations?

(4) What is the critical LDL concentration below which IHD is unlikely to occur, no matter how low the HDL concentration is?

In the present study only the relationship between the serum lipoprotein profile and the presence or absence of CAD was investigated. No attempt was made to correlate the extent of atherosclerotic lesions with the severity of

dyslipoproteinemia. Additional risk factors, such as hypertension, cigarette smoking or obesity were not considered.

Materials and Methods

Blood was drawn after an overnight fast and allowed to clot at room temperature for 60 min. Serum was separated by low speed centrifugation.

Patients were males between the ages of 40 and 60 years, who underwent coronary angiography at the Department of Cardiology at the University Hospital. Survivors of myocardial infarction were excluded from the study if the acute event had taken place less than 6 weeks before examination. In addition we investigated the serum lipid and lipoprotein concentrations in a population of apparently healthy male blood donors aged 18—60 years, the average being 26 years (n = 271).

Lipid determination

Cholesterol and triglycerides were determined using enzymatic tests kits (Boehringer Mannheim, Mannheim, F.R.G.).

Quantitative lipoprotein electrophoresis

Lipoprotein electrophoresis was performed using the "LIPIDOPHOR" system (Immuno GmbH, Vienna, Austria).

Since the results of the present study are dependent on this method, a more detailed description of it is warranted, especially with regard to standardization and precision.

Standardization

The method was originally standardized with highly purified lipoprotein fractions obtained from controls and hyperlipemic patients [8]. Since the relative percentage of the densitometrically measured total integral is used for calculation of the lipoprotein concentrations, expressed as β-, pre-β- and α-lipoprotein cholesterol, only differences in the mass : area integral relationships between the different lipoprotein fractions have to be determined. The lipoprotein concentration can then be calculated from the serum cholesterol concentration, provided the relative cholesterol content of the different lipoprotein fractions is known and fairly constant. From our investigations and from data in the literature [9—11] we derived the following relative cholesterol contents: β-lipoproteins: 45%; pre-β-lipoproteins: 15% and α-lipoproteins: 18%. These data together with the relative consistency of the percentage were recently verified by direct enzymatic determination of the cholesterol content of serum lipoproteins after electrophoretic separation, fixation and densitometric scanning [12].

The directly determined cholesterol concentrations agreed very well with the cholesterol concentrations calculated from densitometry. The standardization was further verified by ultracentrifugation [13] and immunonephelometric determination of the A-I content of corresponding sera using rate nephelometry [14].

Precision of the method

The coefficient of variation (CV) for quantification of β-, pre-β- and α-lipoproteins in a series of 20 cases varied between 2 and 4% [13]. The day-to-day precision over a period of 10 days using a lyophilized control serum (Immuno GmbH, Vienna, Austria) gave a CV of 5% for α- and β-lipoproteins and 7.8% for pre-β-lipoproteins.

Coronary angiography

Coronary angiography was performed accroding to Sones [15] in combination with cineangiography. The presence of CAD was established if one major coronary artery was occluded more than 50%. Patients were classified as free of CAD if none of the coronary arteries were affected.

Results

From a total of 181 patients, 59 did not suffer from CAD (group I), although 3 had a history of myocardial infarction. 122 patients were found to have CAD (group II). 66 of group II patients were survivors of myocardial infarction. The means of the lipid- and lipoprotein concentrations determined in the sera of the two groups of patients are shown in Table 1. The differences between the two groups are most pronounced in the serum cholesterol and β-lipoprotein cholesterol concentrations and in the β-lipoprotein/α-lipoprotein ratio. Rather slight differences are observed in the serum triglyceride concentrations and in the concentrations of pre-β- and α-lipoprotein.

To establish the cut-off points, the percentage of patients in each group which exhibited cholesterol, triglyceride or lipoprotein concentrations beyond a certain critical value was determined (Figs. 1—6). It is apparent that as the critical value increases the percentage of each group correspondingly falls. As shown in Fig. 1, only 3% of patients suffering from CAD (group II) have serum cholesterol values below 170 mg/100 ml. Such values may therefore be considered to be associated with a low risk for CAD. On the other hand only 5% of patients free of CAD (group I) show serum cholesterol levels above 280 mg/100 ml, the concentration range obviously indicating a high risk for CAD. Any serum cholesterol concentration within these limits needs further interpretation in terms of lipoprotein concentrations.

TABLE 1

MEAN SERUM LIPID AND LIPOPROTEIN-CHOLESTEROL CONCENTRATIONS IN PATIENTS WITH (GROUP II) AND WITHOUT (GROUP I) CORONARY ARTERY DISEASE AS SHOWN BY CORONARY ANGIOGRAPHY

	Group I (n = 59)	Group II (n = 122)	Significance
Serum cholesterol (mg/100 ml)	201	236	$P < 0.0001$
Serum triglycerides (mg/100 ml)	176	185	$P < 0.05$
β-Lipoprotein cholesterol (mg/100 ml)	140	174	$P < 0.0001$
Pre-β-lipoprotein cholesterol (mg/100 ml)	29	27	NS
α-Lipoprotein cholesterol	35	34	NS
β-Lipoprotein/α-lipoprotein ratio	1.76	2.3	$P < 0.0001$

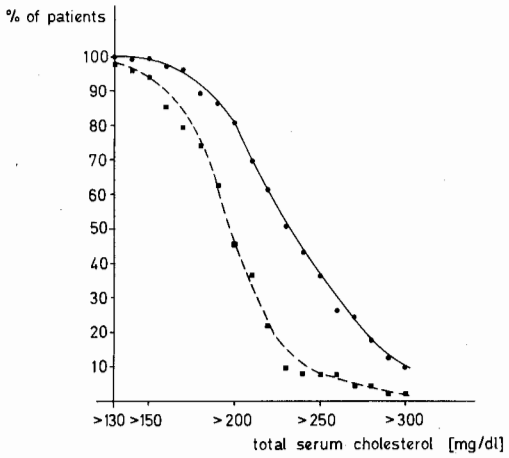

Fig. 1. Difference in serum cholesterol concentrations between Group I (■— — —■) and Group II (●————●) expressed as percentage of subjects exceeding a certain concentration.

Similarly, one can attribute a low risk to β-lipoprotein cholesterol values below 120 mg/100 ml (Fig. 3) and a β-lipoprotein/α-lipoprotein ratio below 1.1 (Fig. 6), and a high risk to β-lipoprotein cholesterol values above 180 mg/100 ml (Fig. 3) and to β-lipoprotein/α-lipoprotein ratios exceeding 2.7 (Fig. 6).

For each lipid or lipoprotein parameter a critical value can be found at which the percentages of the two groups of patients exceeding that critical value show maximum differences. These critical values may be considered as cut-off points. Such points are clearly found for serum cholesterol at 230 mg/100 ml (Fig. 1, difference: 41%) for β-lipoprotein cholesterol at 140 mg/100 ml (Fig. 3, difference: 39%) and for the β-lipoprotein/α-lipoprotein ratio at 1.6 (Fig. 6, difference: 31%). Serum triglycerides, pre-β-lipoprotein cholesterol and α-lipoprotein cholesterol do not exhibit such clear cut-off points (Figs. 2,4 and 5).

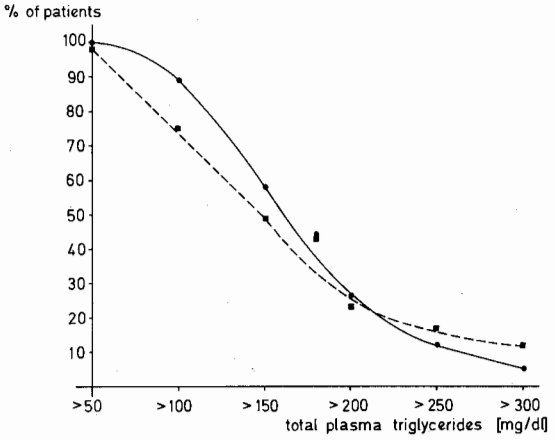

Fig. 2. Difference in serum triglyceride concentrations between Group I (■— — —■) and Group II (●————●) expressed as percentage of subjects exceeding a certain concentration.

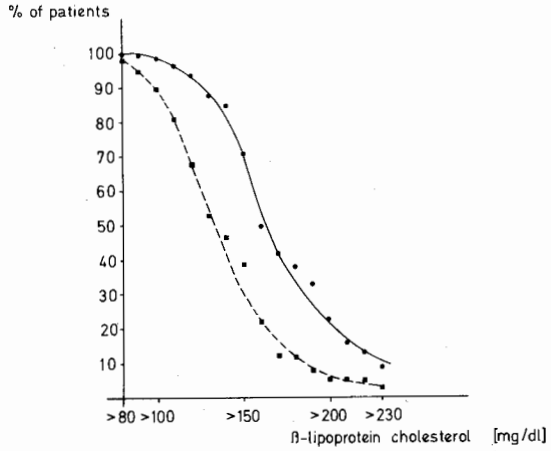

Fig. 3. Difference in β-lipoprotein cholesterol concentrations between Group I (■— — —■) and Group II (●————●) expressed as percentage of subjects exceeding a certain concentration.

A combination of these critical values (Combination 1, Table 2) (serum cholesterol 230 mg/100 ml, β-lipoprotein cholesterol 140 mg/100 ml and β-lipoprotein/α-lipoprotein ratio >1.6) provides a cut-off point enabling differentiation of up to 50% between Group I and Group II, provided that at least 2 of these critical values are exceeded. In this case a patient is classifiable as a risk-patient.

A similar discrimination (up to 49%) can be reached using a β-lipoprotein cholesterol concentration of 155 mg/100 ml, independent of the β-lipoprotein/α-lipoprotein ratio, together with a β-lipoprotein cholesterol concentration of 135 mg/100 ml in combination with a β-lipoprotein/α-lipoprotein ratio>1.6 as cut-off points (Combination 2, Table 2). Using the latter criteria, 37% of Group I patients are incorrectly classified, whereas 86% of Group II patients are recognized (Table 2). Replacement of the β-lipoprotein/α-lipoprotein ratio of

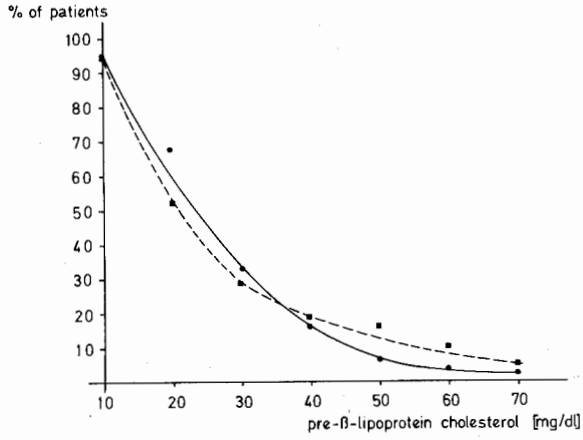

Fig. 4. Difference in pre-β-lipoprotein cholesterol concentrations between Group I (■— — —■) and Group II (●————●) expressed as percentage of subjects exceeding a certain concentration.

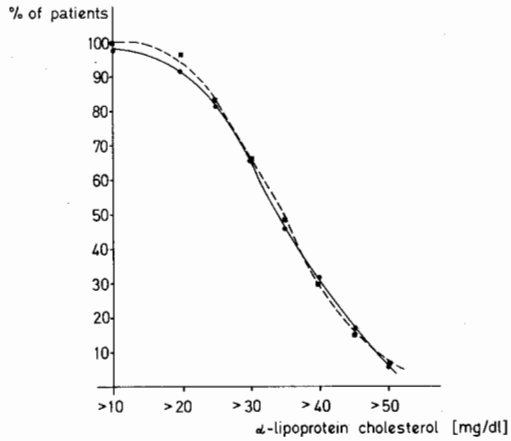

Fig. 5. Difference in α-lipoprotein cholesterol concentrations between Group I (■— — —■) and Group II (●————●) expressed as percentage of subjects exceeding a certain concentration.

>1.6 as critical value by an α-lipoprotein cholesterol concentration of less than 35 mg/100 ml (Combination 3, Table 2), results in a less efficient differentiation.

Using Combination 1 as the most effective, the patients can be divided into subgroups A—F (Table 3). Patients falling into subgroups A—C are classified as risk patients.

The lipid and lipoprotein concentrations of healthy blood donors (age 18—60 years, mean: 26, n = 271) are presented in Table 4. Only 10% of these can be classified as being at risk according to the criteria of Combination 1. Almost all of these risk patients have possibility B (Table 3) of Combination 1.

There were no striking differences in the average α-lipoprotein cholesterol concentrations found in the two groups of patients. However inclusion of the

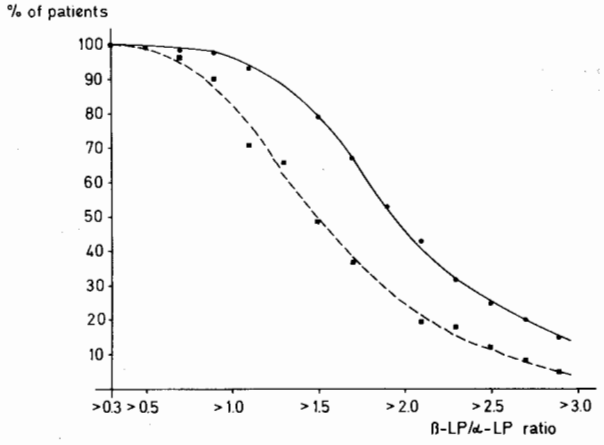

Fig. 6. Difference in β-lipoprotein/α-lipoprotein ratio between Group I (■— — —■) and Group II (●————●) expressed as percentage of subjects exceeding a certain value.

TABLE 2

COMBINATION OF LIPID AND LIPOPROTEIN PARAMETERS SERVING AS CUT-OFF POINTS

Each parameter represents a critical value which has to be exceeded (with exception of the α-lipoprotein cholesterol in Combination 3). In Combinations 1 and 3 at least two critical values have to be exceeded for categorization for risk of CAD.

	Group I	Group II
Combination 1	31%	80%
Serum cholesterol > 230 mg/100 ml		
β-lipoprotein cholesterol > 140 mg/100 ml		
β-lipoprotein/α-lipoprotein ratio > 1.6		
Combination 2	37%	86%
β-lipoprotein cholesterol > 155 mg/100 ml		
or β-lipoprotein cholesterol > 135 mg/100 ml		
+ β-lipoprotein/α-lipoprotein ratio > 1.6		
Combination 3	33%	75%
Serum cholesterol > 230 mg/100 ml		
β-lipoprotein cholesterol > 140 mg/100 ml		
α-lipoprotein cholesterol < 35 mg/100 ml		

TABLE 3

DISTRIBUTION OF ALL PATIENTS OVER ALL POSSIBLE COMBINATIONS OF CRITICAL VALUES ACCORDING TO COMBINATION 1

Fulfilling the criteria of possibilities A—C leads to classification as risk-patient.

	Group I	Group II
A: Serum cholesterol > 230 mg/100 ml + β-lipoprotein cholesterol > 140 mg/100 ml + β-lipoprotein/α-lipoprotein ratio > 1.6	12%	40%
B: Serum cholesterol > 230 mg/100 ml + β-lipoprotein cholesterol > 140 mg/100 ml + β-lipoprotein/α-lipoprotein ratio <1.6	0%	12%
C: β-lipoprotein cholesterol > 140 mg/100 ml + β-lipoprotein/α-lipoprotein ratio > 1.6	19%	29%
D: Serum cholesterol < 230 mg/100 ml + β-lipoprotein cholesterol > 140 mg/100 ml + β-lipoprotein/α-lipoprotein ratio < 1.7	14%	6%
E: Serum cholesterol < 230 mg/100 ml + β-lipoprotein cholesterol < 140 mg/100/ml + β-lipoprotein/α-lipoprotein ratio > 1.6	11%	6%
F: Serum cholesterol < 230 mg/100 ml + β-lipoprotein cholesterol < 140 mg/100 ml + β-lipoprotein/α-lipoprotein ratio ⩽ 1.7	44%	7%

TABLE 4

MEAN LIPID AND LIPOPROTEIN CHOLESTEROL CONCENTRATIONS IN NORMAL CONTROLS (n = 271) 18—60 YEARS OF AGE, THE MEAN BEING 26

Substance	Concentration (mg/100 ml)
Serum cholesterol	175
Serum triglycerides	98
β-lipoprotein cholesterol	104
Pre-β-lipoprotein cholesterol	17
α-lipoprotein cholesterol	50
β-lipoprotein/α-lipoprotein ratio	0.86

TABLE 5

AVERAGE α-LIPOPROTEIN CHOLESTEROL CONCENTRATIONS OF GROUP I AND GROUP II DIVIDED INTO SUBGROUPS ACCORDING TO THE SERUM CHOLESTEROL CONCENTRATION

Serum cholesterol	Mean α-cholesterol (mg/100 ml)	
	Group I	Group II
>280	28 (n = 3)	41 (n = 21)
230—280	34 (n = 6)	35 (n = 40)
200—230	33 (n = 19)	32 (n = 37)
180—200	38 (n = 16)	32 (n = 10)
160—180	38 (n = 8)	31 (n = 10)
140—160	29 (n = 5)	22 (n = 4)

β-lipoprotein/α-lipoprotein ratio as a criterion for differentiation improved the separation markedly, We therefore compared the mean α-lipoprotein cholesterol concentrations in subgroups of Groups I and II, adjusted for the concentrations of serum cholesterol and β-lipoprotein cholesterol (Tables 5 and 6).

At serum cholesterol concentrations above 200 mg/100 ml no striking differences can be seen. Differences are most pronounced at serum cholesterol levels between 160 and 200 mg/100 ml and β-lipoprotein cholesterol levels between 130 and 160 mg/100 ml.

Discussion

Our present knowledge about the role and possible antagonistic function of the major lipoprotein fractions in atherogenesis justifies great emphasis on the improvement of our analytical methodology in this field of clinical research.

Large-scale epidemiological studies, even with less refined methods for lipoprotein quantification, can provide very useful information regarding the basic mechanisms of atherogenesis. However application of such information for the individual assessment of the risk of coronary artery disease (CAD) is difficult and often impossible.

TABLE 6

AVERAGE α-LIPOPROTEIN CHOLESTEROL CONCENTRATIONS OF GROUP I AND GROUP II DIVIDED INTO SUBGROUPS ACCORDING TO THE β-LIPOPROTEIN CHOLESTEROL CONCENTRATION

β-lipoprotein cholesterol (mg/100 ml)	Mean α-cholesterol (mg/100 ml)	
	Group I	Group II
>190	32 (n = 3)	35.4 (n = 37)
160—190	34 (n = 10)	36.3 (n = 32)
130—160	35.4 (n = 21)	31.9 (n = 38)
100—130	36.6 (n = 18)	34.8 (n = 9)
<100	36 (n = 7)	20 (n = 2)

Quantitative lipoprotein electrophoresis based on densitometric scanning of lipoprotein bands separated by agarose gel electrophoresis and visualized in the gel by polyanion precipitation has proved to be a very reproducible and precise method [13], which is relatively easy to perform. It allows direct estimation of the concentration of the β-lipoproteins or LDL and is especially suited for the determination of the concentration ratios of the different serum lipoproteins, since the concentrations are determined after a single pipetting step. This pipetting step does not have to be accurate because measurement of lipoprotein bands is based on the relative percentage of a total integral. We applied this very precise method for quantification of serum lipoproteins to the analysis of serum of patients with and without CAD as shown by coronary angiography. With this method we tried to find a single lipoprotein parameter or a combination of lipid and lipoprotein concentrations indicative of CAD in the corresponding patient. Since the major purpose of this study was to investigate the connection between concentrations of serum lipoproteins and atherogenesis we confined ourselves to the assessment of serum lipoproteins irrespective of other risk factors.

The indicative value of a parameter was studied by its ability to differentiate between two groups of patients: Group I with no sign of CAD, Group II with CAD, as judged by angiography. Group I of course does not represent a population of normal controls, but consists of patients with signs or symptoms of cardiac disease. The marked differences in lipid and lipoprotein concentration as compared to the group of normal controls (Table 4) may reflect this fact.

The two best single parameters for differentiation are the concentrations of serum cholesterol or β-lipoprotein cholesterol, followed by the β-lipoprotein/α-lipoprotein ratio. Triglycerides, pre-β-lipoproteins and α-lipoproteins allowed no satisfactory separation. At the critical values of maximum separation, however, both serum cholesterol (230 mg/100 ml, Group II) and β-lipoprotein cholesterol (140 mg/100 ml, Group I) divide the corresponding groups approximately at the 50% level (Figs. 1 and 3), and therefore cannot be considered as very useful cut-off points.

Introduction of the β-lipoprotein/α-lipoprotein ratio as an additional parameter (Combination 1, Table 2) shifts the range of differentiation (31% vs. 81%) favorably and increases the rate of differentiation by about 10%. Combination 1 clearly provides the best cut-off points and is derived from Figs. 1, 3 and 6. A risk for CAD is anticipated if at least two of the three critical values of Combination 1 are exceeded. This is the case in 81% of Group II patients vs. 31% of Group I patients, which is the maximum separation so far obtained, based on lipoprotein quantification alone. Combination 2 yields a similar differentiation to that achieved with Combination 1 (Table 2), but the percentage of group I patients categorized as risk patients is much closer to the 50% level, reaching 37%. Combination 2 suggests that α-lipoproteins may exert their antiatherogenic role only within a certain range of β-lipoprotein concentrations (β-lipoprotein cholesterol concentration between 135 and 155 mg/100 ml, see Tables 5 and 6).

Introduction of the β-lipoprotein/α-lipoprotein ratio in Combination 1 or 2 allows detection of an additional 29% of Group II patients. With this ratio it is

possible to detect risk patients showing a "normal" serum cholesterol concentration with a simultaneous relatively high β-lipoprotein concentration accompanied by a low α-lipoprotein concentration. This condition may be termed dyslipoproteinemia. As indicated above and extended further below in this particular condition, which is not yet present to a large extent in the relatively young normal controls (Table 4), a low concentration of α-lipoproteins apparently contributes to the development of CAD.

Replacement of the β-lipoprotein/α-lipoprotein ratio in Combination 1 by α-lipoprotein cholesterol (cut-off point: α-lipoprotein cholesterol <35 mg/100 ml, Combination 3, Table 2) decreases the percentage of recognized risk patients from 81% to 75%. This fact and the low differentiating ability of the α-lipoprotein cholesterol concentration (Fig. 5) is surprising in view of the improvement in differentiation brought about by introduction of the β-lipoprotein/α-lipoprotein ratio. In 22% of Group II patients, this ratio is increased to over 1.6 but they still have an α-lipoprotein cholesterol concentration exceeding 35 mg/100 ml. In order to find an explanation for the discriminating ability of the ratio, the average α-lipoprotein cholesterol concentrations were calculated in subgroups of Groups I and II according to corresponding serum cholesterol and β-lipoprotein cholesterol levels (Tables 5 and 6), i.e. adjusted for these parameters. Major differences in the α-lipoprotein cholesterol concentrations between the two groups of patients were found in the concentration ranges of 160—200 mg/100 ml for serum cholesterol and 130—160 mg/100 ml for β-lipoprotein cholesterol. Above these ranges groups I and II show no differences in the α-lipoprotein cholesterol concentrations. Since 81% of group II patients show serum-cholesterol concentrations above 200 mg/100 ml, low α-lipoprotein concentrations in this group of patients can therefore not be considered as a major contributory factor in the development of CAD.

The incidence rate of CAD is low at serum cholesterol concentrations below 170 mg/100 ml (Fig. 1) and at β-lipoprotein cholesterol concentrations below 130 mg/100 ml (Fig. 3). The range of β-lipoprotein concentrations within which α-lipoproteins may exert their possible antiatherogenic function is found to be between 130 and 160 mg/100 ml β-lipoprotein cholesterol (31% of Group II patients). This may mean, that at a β-lipoprotein cholesterol concentration exceeding 160 mg/100 ml, the possible protective effect of α-lipoproteins is overruled, whereas at γ-lipoprotein cholesterol concentrations below 130 mg/100 ml, the risk for CAD is markedly diminished. In this case low levels of α-lipoproteins would result in low serum cholesterol levels.

From Table 5 one might conclude that lower α-lipoprotein cholesterol values in Group II at serum cholesterol levels below 200 mg/100 ml are counterbalanced by high β-lipoprotein cholesterol concentrations. In this case the low α-lipoprotein concentration would mask a hyper-β-lipoproteinemia. Table 6, however, shows that at the same β-lipoprotein cholesterol levels, Group II patients on average have lower α-lipoprotein cholesterol concentrations, possibly indicating a lack of protection against CAD. Differences in the α-lipoprotein concentrations of the two groups of patients are possibly masked by the large number of patients in Group II having serum cholesterol concentrations exceeding 200 mg/100 ml.

Although this study is not a prospective one, in our opinion these data show

the usefulness of a very precise method for quantification of serum lipoproteins for assesssing the risk of CAD in a single patient. The results of this study show that neither the determination of serum cholesterol concentration nor that of α-lipoprotein cholesterol concentration alone is adequate. Accurate determination of the β-lipoprotein cholesterol concentration and of the β-lipoprotein/α-lipoprotein ratio together with accurate estimation of the serum cholesterol concentration are essential for an assessment of the risk of CAD in a single patient. The outcome of this study which at present is being continued and extended supports the close connection between serum lipoproteins and atherogenesis. This study illustrates that meaningful clinical investigation and development of methodology for investigation of lipoprotein metabolism is dependent on the knowledge of some basic mechanisms of lipoprotein metabolism derived from in vitro studies on a cellular basis. As long as the clinical chemical methodology keeps pace with basic research even better possibilities for early recognition of risk of CAD based on investigation of plasma lipoproteins may be expected in the near future.

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