J. Clin. Chem. Clin. Biochem.
Vol. 24, 1986, pp. 627–635

HDL Apolipoprotein A-I and HDL Apolipoprotein A-II Concentrations in Male Company Employees in Westphalia Aged 40 Years and Older

By *H. Schriewer, F. Emke, H. Funke*

Institut für Klinische Chemie und Laboratoriumsmedizin (Zentrallaboratorium) Medizinische Einrichtungen der Westfälischen Wilhelms-Universität Münster/Westfalen

H. Schulte

Institut für Arteriosklerosеforschung an der Universität Münster, Münster/Westfalen and

G. Assmann

Institut für Klinische Chemie und Laboratoriumsmedizin (Zentrallaboratorium) Medizinische Einrichtungen der Westfälischen Wilhelms-Universität Münster/Westfalen

(Received January 10, 1985/January 2/April 30, 1986)

Summary: The major structural components of high density lipoproteins were determined in the sera of 638 male employees aged 40 years and older. It was demonstrated that the HDL apolipoprotein A-I/HDL cholesterol ratio as well as the HDL apolipoprotein A-II/HDL cholesterol ratio are similarly correlated to a cumulative score of established risk factors for atherosclerosis. Most important, however, is the finding that the correlation of these ratios to the risk factor rating of atherosclerosis is found in subgroups with normal or elevated HDL cholesterol values. Furthermore, it is shown that the relative content of apolipoproteins A-I and A-II in individual HDL is partly dependent on the plasma concentration of HDL cholesterol and triglycerides.

It is concluded that HDL composition may have an additional predictive significance for the development of atherosclerosis.

HDL-Apolipoprotein A-I- und HDL-Apolipoprotein A-II-Konzentrationen bei männlichen Betriebsangehörigen in Westfalen im Alter von 40 Jahren und darüber

Zusammenfassung: Im Serum von 638 männlichen Betriebsangehörigen im Alter von 40 Jahren und darüber wurden die Hauptstrukturkomponenten der "high density" Lipoproteine untersucht. Es wird gezeigt, daß sowohl das HDL-Apolipoprotein A-I/HDL-Cholesterin-Verhältnis als auch das HDL-Apolipoprotein A-II/HDL-Cholesterin-Verhältnis vergleichbar mit einem kumulativen Score von bekannten Risikofaktoren der Atherosklerose korreliert ist. Am bedeutsamsten ist die Beobachtung, daß die Korrelation zwischen diesen Quotienten und dem Risiko-Score in Untergruppen mit normalen und erhöhten HDL-Cholesterinwerten erhalten bleibt. Darüberhinaus wird gezeigt, daß der relative Anteil von Apolipoprotein A-I und Apolipoprotein A-II in den HDL teilweise von der Konzentration von HDL-Cholesterin und Triglyceriden im Plasma abhängt.

Die Ergebnisse deuten darauf hin, daß der HDL-Komposition eine zusätzliche prädiktive Bedeutung für die Entwicklung der Atherosklerose zukommt.

Introduction

In recent epidemiological (1–4) and clinical studies (5–6) the analysis of high density lipoprotein cholesterol (HDL cholesterol) has been shown to be a risk indicator for coronary heart disease. In contrast to HDL cholesterol, the possible relationship of HDL apolipoproteins to the risk of coronary heart disease has not yet been thoroughly investigated. Several clinical studies have demonstrated low levels of apolipoprotein A-I in subjects with coronary heart disease (7–9), while apolipoprotein A-II in patients with coronary heart disease has been found to be low (10, 11) or unchanged (6, 8). In contrast to these clinical studies there is a lack of the prospective epidemiological data needed to demonstrate the predictive potency of HDL apolipoproteins with respect to coronary heart disease. Recently, from initial results of our epidemiological study in company employees in Westphalia, it was demonstrated that HDL apolipoprotein A-I correlates with several risk factors for coronary heart disease but fails to correlate with the risk factors triacylglycerol and relative body weight (12). To date, no comparable studies on HDL apolipoprotein A-II are available. For a greater insight into the relationship between HDL apolipoproteins and the risk factors for coronary heart disease, we measured both HDL apolipoproteins A-I and A-II in 638 male company employees in addition to HDL cholesterol. Since the incidence of coronary heart disease in women and in men under 40 years of age is very low, our study was limited to individuals aged 40 years and above.

Materials and Methods

Sample material

Sera were obtained from male employees aged 40 years and over. All of these were participants in the "Prospective epidemiological study of company employees in Westphalia" (13). Data given here are cross sectional. The proband's mean age was 49 ± 6 years, the maximum age being 64 years; observed body weight expressed as *Broca* index ranged from 69% to 151% with a mean value of 106.6% ± 12.2%.

Analysis of HDL components

HDL components were analysed in the supernatant after precipitation of apolipoprotein B-containing lipoproteins with phosphotungstic acid/$MgCl_2$ using the Boehringer Mannheim Test Combination (test no. 1442434) (14). HDL cholesterol was analysed enzymatically using the CHOD-PAP method (Boehringer Mannheim, test combination nol 237574) (14). HDL phosphatidyl choline was determined with an enzymatic colour test using the centrifugal analyser Cobas Bio (Hoffmann La Roche) as described previously (15).

Apolipoprotein A-I was determined turbidimetrically using the Cobas Bio analyser.

The incubation mixture contained:

- 300 µl phosphate-buffered polyethyleneglycol solution (Immunochemical System Beckman)
- 20 µl diluted sample (dilution 1 : 160 with 9 g/l NaCl)
- 20 µl antibody solution (Behring antibody no. 103315 B was diluted with 1 : 2.5 with 9 g/l NaCl)
- 10 µl H_2O

As a blank, 300 µl phosphate-buffered polyethyleneglycol solution was used, containing 20 µl diluted sample (dilution 1 : 160 with 9 g/l NaCl) and 10 µl H_2O. After an incubation period at 25 °C changes in turbidity were recorded 30 times. For calibration, a standard solution of known concentration (Behring Apolipoprotein A Standard Serum Lot No. A 041206 K) was diluted 1 : 30, 1 : 60, 1 : 120, 1 : 240, and 1 : 480 with 9 g/l NaCl.

The parameter list for the Cobas Bio reads as follows:

1	unit	8 (mg/dl)
2	calculation factor	1000
3	standard 1 conc	23.1
3	standard 2 conc	46.2
3	standard 3 conc	92.4
3	standard 4 conc	184.8
3	standard 5 conc	369.6
6	limit	0
7	temperature (°C)	25.0
8	type of analysis	7.6
9	wavelength (nm)	340
10	sample dilution (µl)	20
11	diluent volume (µl)	20
12	reagent volume (µl)	300
13	incubation time (s)	10
14	start reagent volume (µl)	20
15	time of first reading (s)	1.0
16	time interval (s)	10
17	number of readings	30
18	blanking mode	1
19	printout mode	1

Using this turbidimetric method identical results were obtained when compared with data observed using kinetic nephelometry (16).

HDL apolipoprotein A-II was measured by turbidimetry using the Cobas Bio analyser as described in detail elsewhere (17).

LDL cholesterol

LDL cholesterol was calculated using the *Friedewald* method (18).

Total cholesterol, triacylglycerol, uric acid

Serum levels of cholesterol, triacylglycerol, glucose and uric acid were determined with the SMAC II Analyser (Technicon GmbH, Bad Vilbel, FRG) as described elsewhere (13).

Statistics

For statistical calculations we used the statistical package for the social sciences (SPSS). To describe relationships to risk factors *Spearman*'s correlation coefficients were chosen since the risk factors were not always normally distributed, as proved by the *Kolmogorov-Smirnow* test. The correlations of apolipoproteins to the coronary risk score were calculated by *Kendall*'s τ because of the high number of tied ranks. Differences in the distribution between subgroups were tested by the *Kruskal-Wallis* test. The level of significance was set at 0.05.

Results

Distribution of HDL apolipoprotein A-I and A-II

Figure 1 shows the HDL apolipoprotein A-I values measured in company employees 40 years and older. The parameters of distribution were:
mean: 1.444 g/l,
S. D.: ± 0.222 g/l,
median: 1.435 g/l,
minimum 0.75 g/l,
maximum 2.65 g/l,
n = 617.

The corresponding values of apolipoprotein A-II are shown in figure 2:
mean: 0.427 g/l,
S. D.: ± 0.073 g/l,
median: 0.423 g/l,
minimum: 0.248 g/l,
maximum: 0.748 g/l,
n = 638.

Correlation to cumulative risk factor rating

To test the relationship between HDL cholesterol, HDL apolipoprotein A-I/HDL cholesterol ratio,

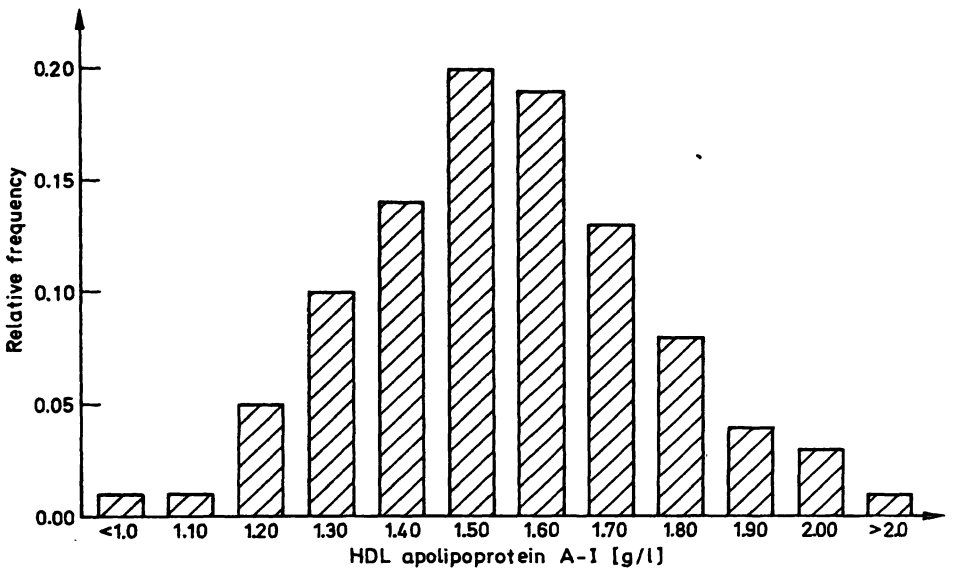

Fig. 1. HDL apolipoprotein A-I values (g/l) in 616 normal male individuals 40 years and older. Epidemiological study of company employees in Westphalia — cross sectional data. On the horizontal axis the upper bounds of the intervals are given.

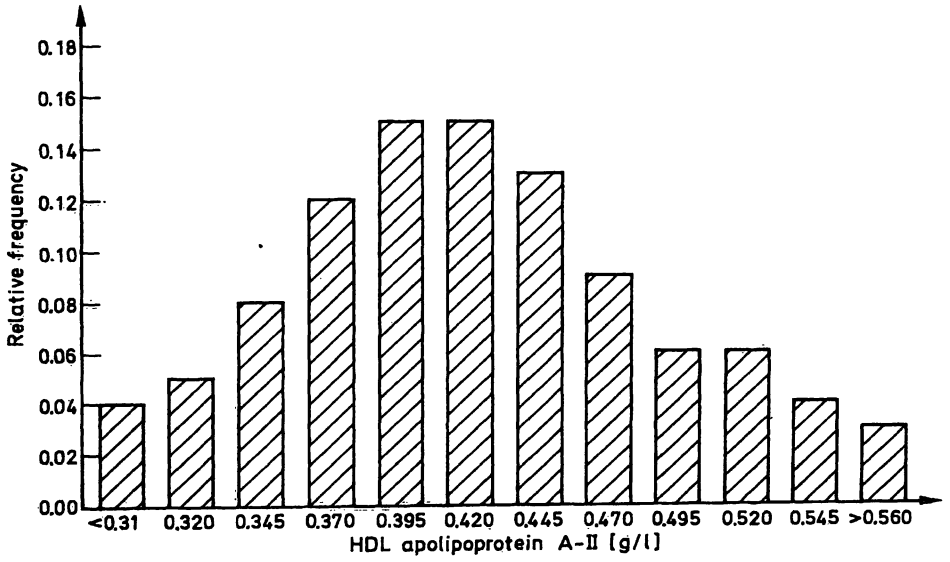

Fig. 2. HDL apolipoprotein A-II values (g/l) in 638 normal male individuals 40 years and older. Epidemiological study of company employees in Westphalia — cross sectional data. On the horizontal axis the upper bounds are given.

HDL apolipoprotein A-II/HDL cholesterol ratio and risk factors, a cumulative risk factor rating was established for each subject. It was based on relative weight, diastolic blood pressure, cigarette smoking, cholesterol value and exercise. A score of 0, 1 or 2 was allocated for each of these measurements (tab. 1) and the sum of these individual scores was taken as the cumulative rating (19). The relation between each of the three parameters and increasing risk score was significant ($p < 0.001$ each). The distribution of the three parameters in the subgroups with a cumulative risk rating score of 0, 1, 2–6 are demonstrated in figures 3, 4 and 5 where additionally the median values are indicated. The differences in the distributions between the subgroups are significant (*Kruskal-Wallis* test, $p < 0.001$ each). The relationship between the HDL apolipoprotein A-I/HDL cholesterol ratio and the cumulative risk rating score was comparable to the relationship of HDL cholesterol and the risk score (tab. 2).

Tab. 1. Risk rating score sheet.

	0 point	1 point	2 points
Relative body weight (*Broca* Index, %)	<100	100–120	>120
Diastolic blood pressure (mm Hg)	< 90	90–110	>110
Cigarette smoking (no./day)	non-smoker	1– 20	> 20
Cholesterol (mmol/)	< 6.72	6.72–7.75	> 7.75
Exercise	very active	slightly active	inactive

An analysis of subgroups with low (< 0.91 mmol/l), normal (0.91–1.40 mmol/l) or elevated (> 1.40 mmol/l) HDL cholesterol values revealed that the two ratios were related to the cumulative risk factor rating in subjects with normal or elevated HDL cho-

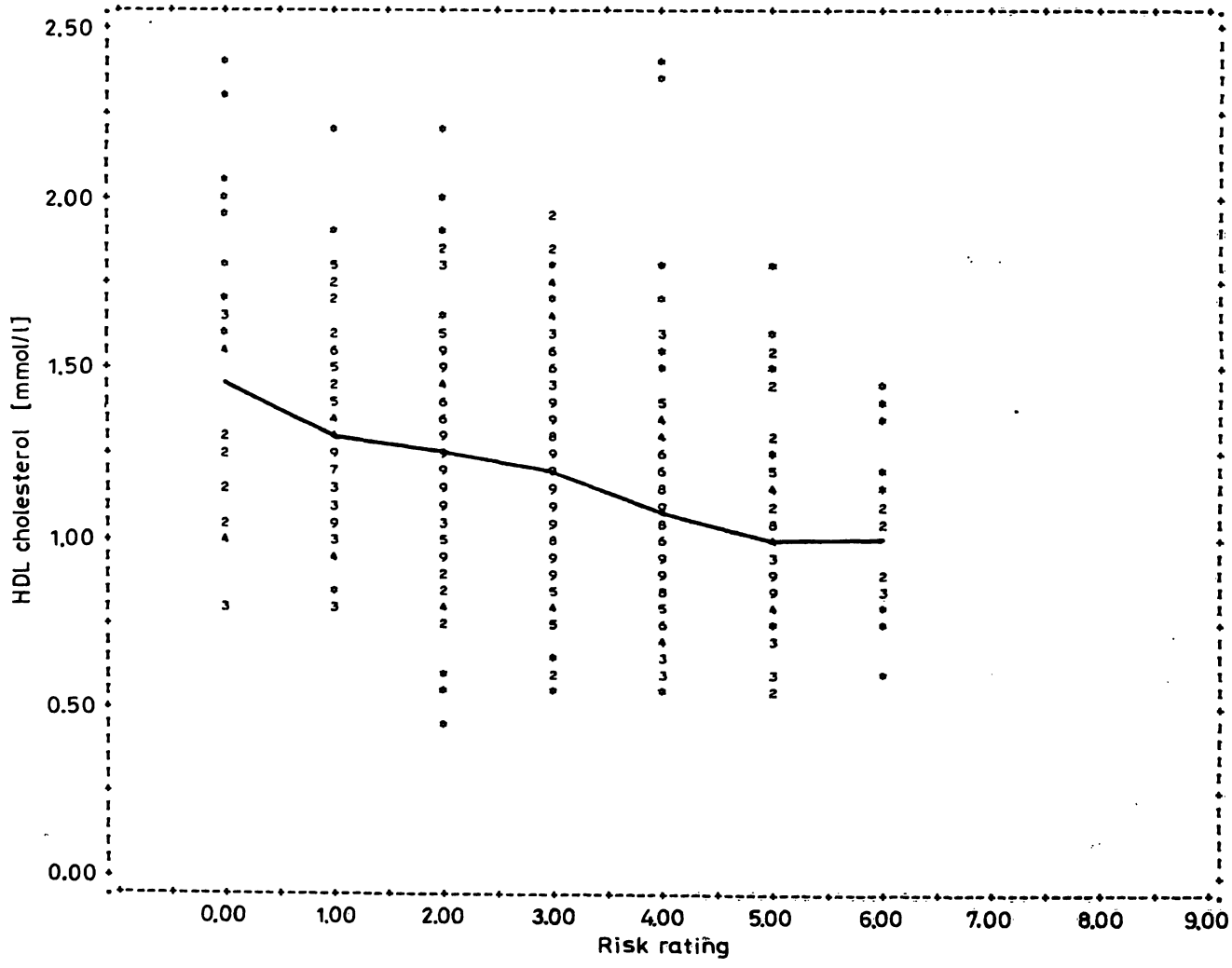

Fig. 3. HDL cholesterol in relation to cumulative risk factor rating. A '9' is printed if 9 or more men have equal values. r (*Kendall*) = −0.280 ($p < 0.001$)

Tab. 2. Correlation coefficients (*Kendall*'s τ) between the coronary risk score and HDL-cholesterol, HDL apolipoprotein A-I/HDL cholesterol, HDL apolipoprotein A-II/HDL cholesterol.

	HDL apolipoprotein A-I/HDL cholesterol	HDL apolipoprotein A-II/HDL cholesterol	HDL cholesterol
HDL cholesterol < 0.907 mmol/l	0.092 n = 112	0.026 n = 114	0.070 n = 114
HDL cholesterol 0.907–1.424 mmol/l	0.260*** n = 369	0.206*** n = 381	−0.109* n = 381
HDL cholesterol > 1.424 mmol/	0.149* n = 135	0.206 n = 142	−0.097 n = 142
Total group	0.300*** n = 616	0.287*** n = 637	−0.280*** n = 637

* $p < 0.05$
** $p < 0.01$
*** $p < 0.001$

lesterol values (tab. 2). Therefore, it seems possible that in subjects with normal or elevated HDL cholesterol values, high ratios of HDL apolipoprotein A-I/HDL cholesterol and HDL apolipoprotein A-II/HDL cholesterol reflects an increased risk of developing coronary heart disease.

HDL apolipoprotein A-I/HDL apolipoprotein A-II ratio

The HDL apolipoprotein A-I/HDL apolipoprotein A-II ratio was positively correlated with HDL cholesterol ($r = 0.194$, $p < 0.001$) (tab. 3, fig. 6). Furthermore, in individuals with low HDL cholesterol values (< 0.907 mmol/l), a relatively low HDL-apolipoprotein A-I/HDL apolipoprotein A-II ratio (3.23 ± 0.47) was calculated. Individuals with high HDL cholesterol values (> 1.41 mmol/l) showed significantly higher HDL apolipoprotein A-I/HDL apolipoprotein A-II ratios (3.67 ± 0.52, $p < 0.001$). The HDL apolipoprotein A-I/HDL apolipoprotein A-II

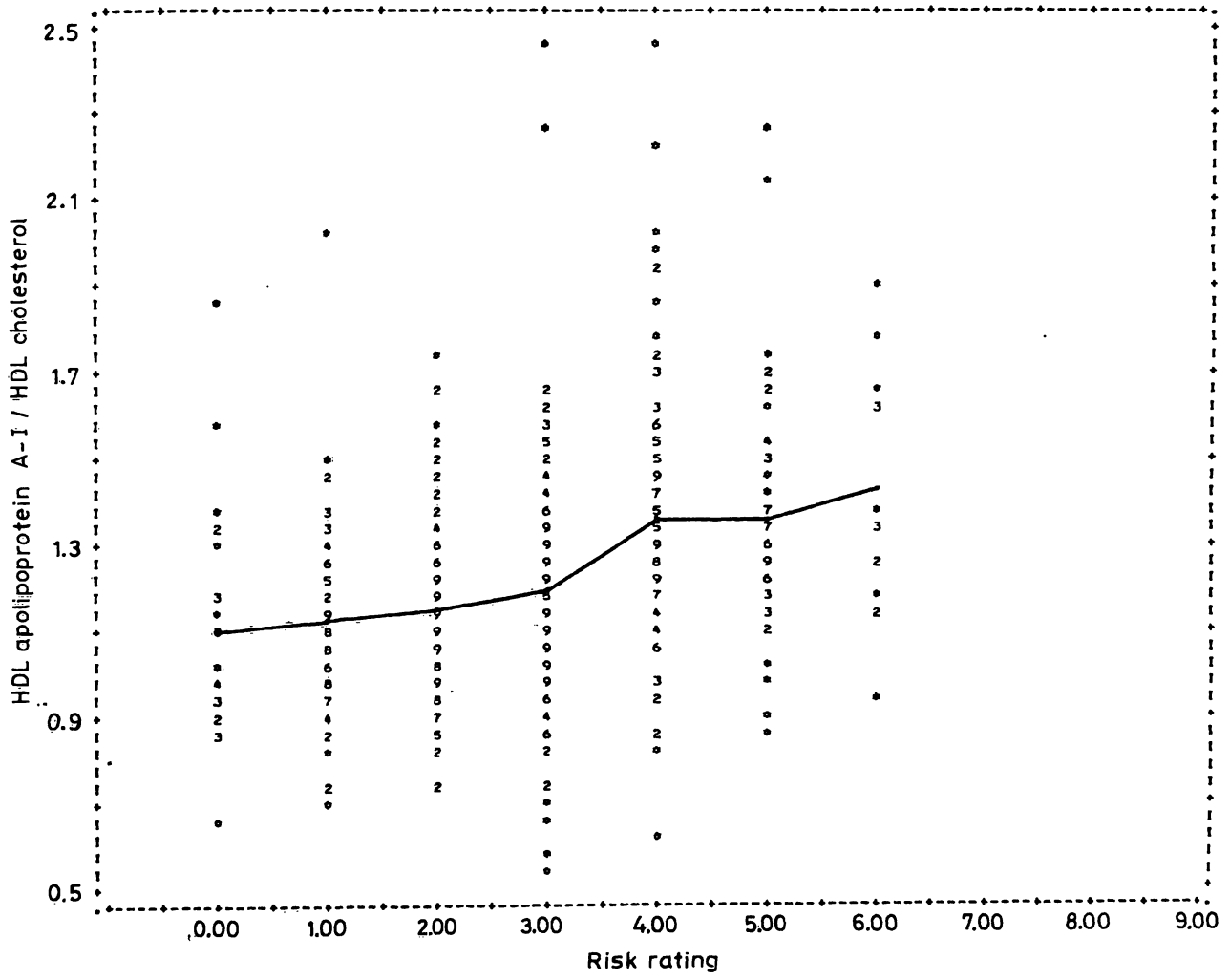

Fig. 4. HDL apolipoprotein A-I/HDL cholesterol ratio in relation to cumulative risk factor rating. A '9' is printed if 9 or more men have equal values.
r (*Kendall*) = −0.287 ($p < 0.001$)

Tab. 3. Correlation coefficients (*Spearman* rank correlation) between the ratio, HDL apolipoprotein A-I/HDL apolipoprotein A-II, and cholesterol, triacylglycerol, HDL cholesterol and HDL phosphatidyl choline.

Cholesterol	−0.061
Triacylglycerol	−0.124*
HDL cholesterol	0.194*
HDL phosphatidyl choline	0.174*

* $p < 0.01$

ratio was negatively correlated with triacylglycerol (r: −0.124, $p < 0.001$) (fig. 7, tab. 3). In individuals with triacylglycerol < 1.71 mmol/l, the HDL apolipoprotein A-I/HDL apolipoprotein A-II ratio was found to be higher (3.51 ± 0.48) than in individuals with triacylglycerol ranging from 1.71 to 2.27 mmol/l (3.33 ± 0.43) or hypertriacylglycerolaemic individuals > 2.27 mmol/l (3.33 ± 0.49). On the other hand, there were no differences in the HDL apolipoprotein A-I/HDL apolipoprotein A-II ratio between hypertriacylglycerolaemic individuals and individuals with medium triacylglycerol values.

Discussion

In our present study we report only data on men 40 years and older, since this group obviously has a higher coronary risk than women or men under 40. With respect to HDL cholesterol and HDL apolipoprotein A-I, the data obtained from men 40 years and older did not differ significantly from data reported previously for company employees from 25 to 64 years old (12). In addition to our previous study, HDL apolipoprotein A-II values were also measured.

As previously reported (19) and as again shown by the present results, HDL cholesterol has a negative correlation to the individual cumulative coronary risk based on the most frequent risk factors, obesity, hypertension, cigarette smoking, cholesterol and exercise. In comparison with HDL cholesterol, the relation of both ratios, HDL apolipoprotein A-I/HDL cholesterol and HDL apolipoprotein A-II/HDL cholesterol, to the individual cumulative risk did not differ. Furthermore, in individuals with normal or elevated HDL cholesterol values a higher coronary

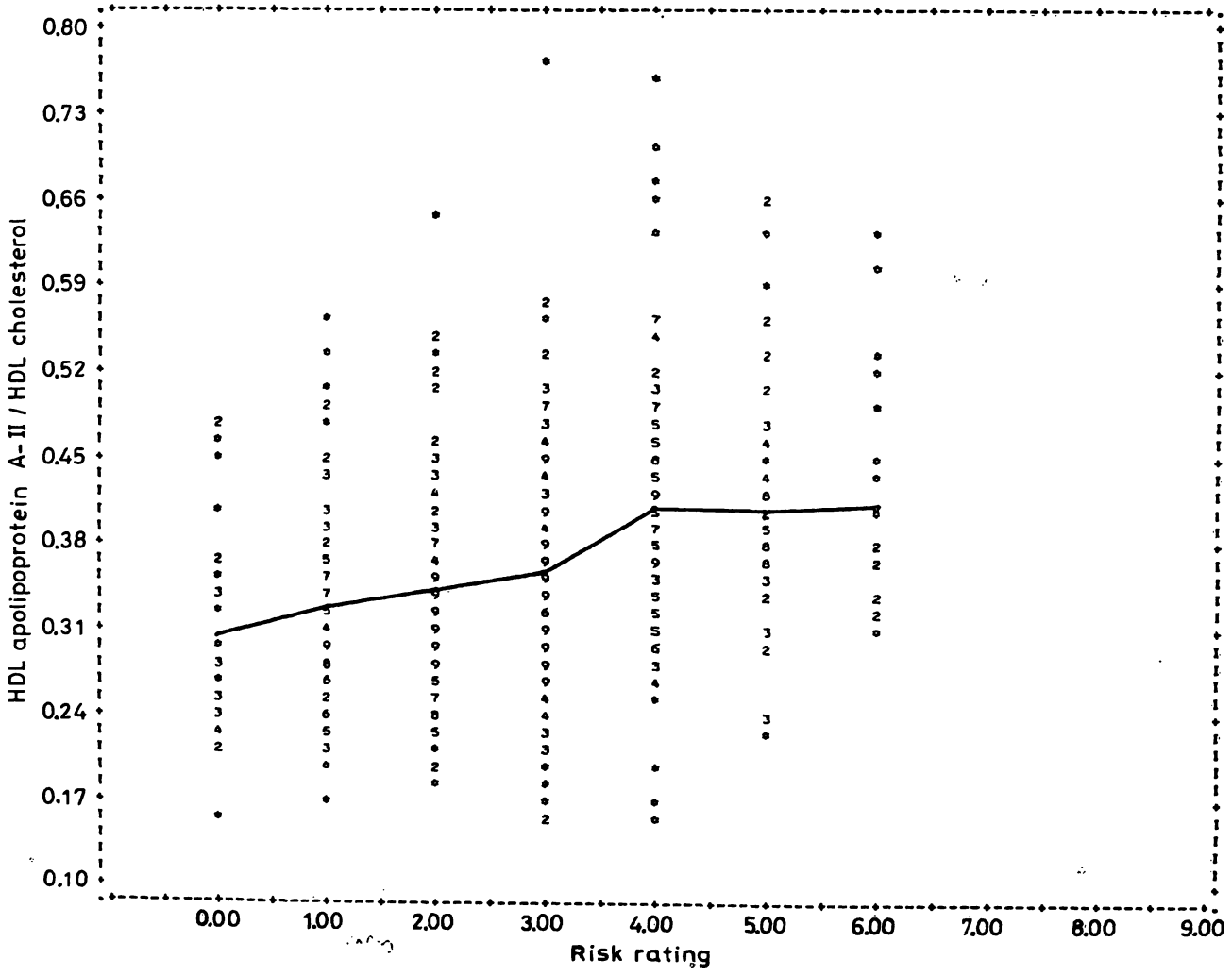

Fig. 5. HDL apolipoprotein A-II/HDL cholesterol ratio in relation to cumulative risk factor rating. A '9' is printed if 9 or more men have equal values.
r (*Kendall*) = 0.300 ($p < 0.001$)

risk was associated with a higher ratio of HDL apolipoprotein A-I/HDL cholesterol and HDL apolipoprotein A-II/HDL cholesterol. The reason for this observation may lie in possible changes in mass and composition of HDL subfractions caused by risk factors and in different associations of HDL subfractions to coronary risk. It is known that changes in plasma HDL level mainly affect HDL_2 (20) and that an inverse correlation exists between HDL and VLDL in plasma (21). Furthermore, it has been shown that a less dense HDL_2 subfraction (relative high lipid/apolipoprotein ratio) is epidemiologically associated with reduced coronary heart disease, while a more dense HDL_3 subfraction (relatively low lipid/apolipoprotein ratio) is thought to be unrelated to such disease (22, 23). Nevertheless, recent studies have shown that HDL in individuals with hypertriacylglycerolaemia mainly consist of HDL_3, which are enriched with apolipoproteins and triglycerides and which are depleted of cholesterol (24). Furthermore, it has been reported that alcohol consumption, which is known to be associated with high HDL cholesterol (25–27) and frequently with hypertriacylglycerolaemia (28, 29), mainly resulted in an enhanced level of HDL_3 mass without affecting HDL_2 mass (30).

It has been shown that the apolipoprotein A-I/apolipoprotein A-II ratio in HDL_2 is higher than that in HDL_3 (31, 32). Therefore, in individuals with heigh HDL cholesterol levels (which are associated with lower triacylglycerol) and a similarly high apolipoprotein A-I/apolipoprotein A-II ratio, a higher HDL_2/HDL_3 ratio may exist in comparison to individuals with lower HDL cholesterol levels (which are associated with higher triacylglycerol) and a similarly low apolipoprotein A-I/apolipoprotein A-II ratio. However, it is of interest to note that the HDL lipoprotein composition in our study did not differ between individuals with moderate hyperlipoproteinaemia and individuals with obviously enhanced lipid levels. Possibly, in hypertriacylglycerolaemics, the HDL apolipoprotein A-I/apolipoprotein A-II ratio may additionally be a reflection of the change in apolipoprotein composition in HDL particles in different density classes.

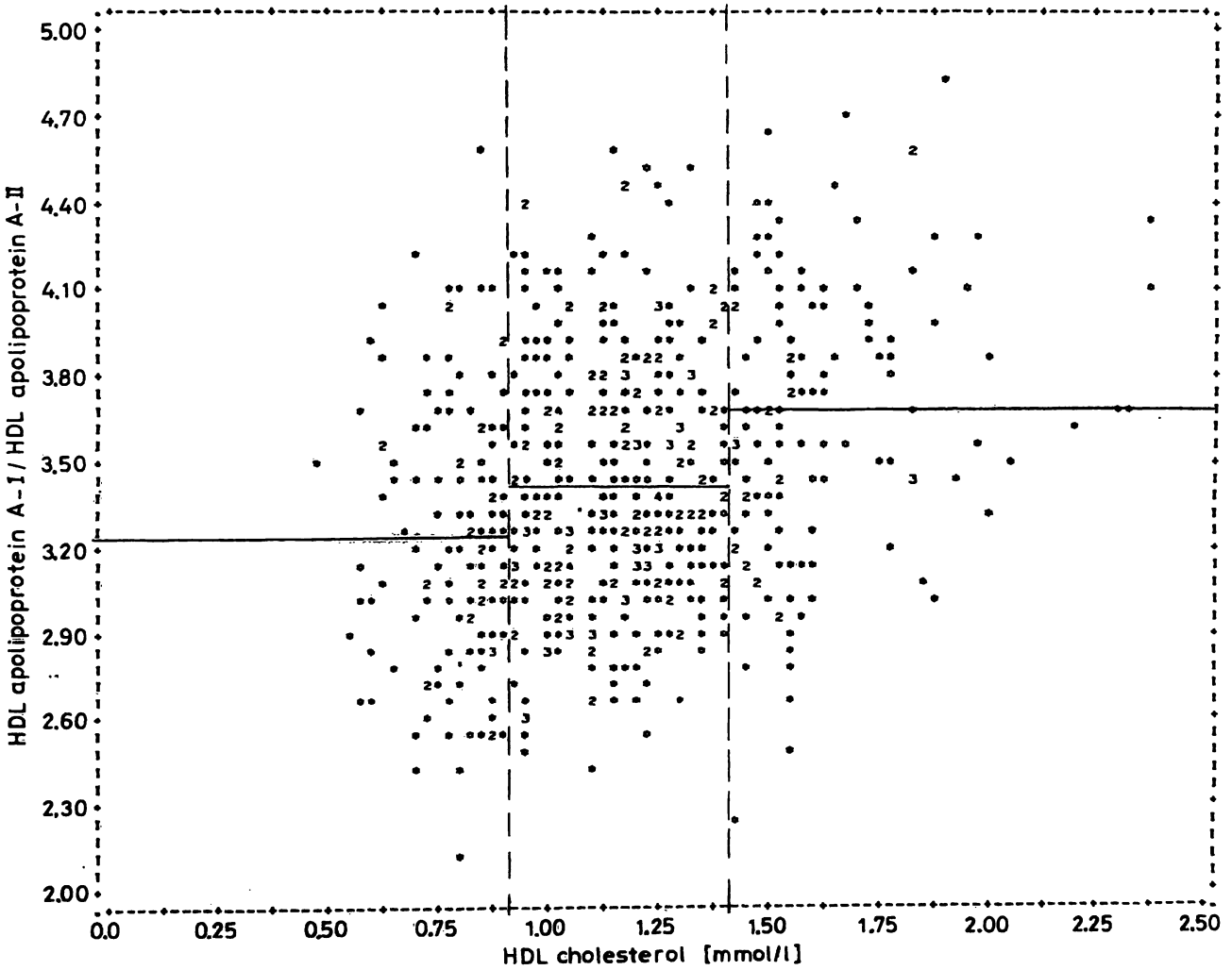

Fig. 6. Bivariate distribution of HDL apolipoprotein A-I/HDL apolipoprotein A-II ratio and HDL cholesterol.
r (*Spearman*) = 0.194 ($p < 0.01$)

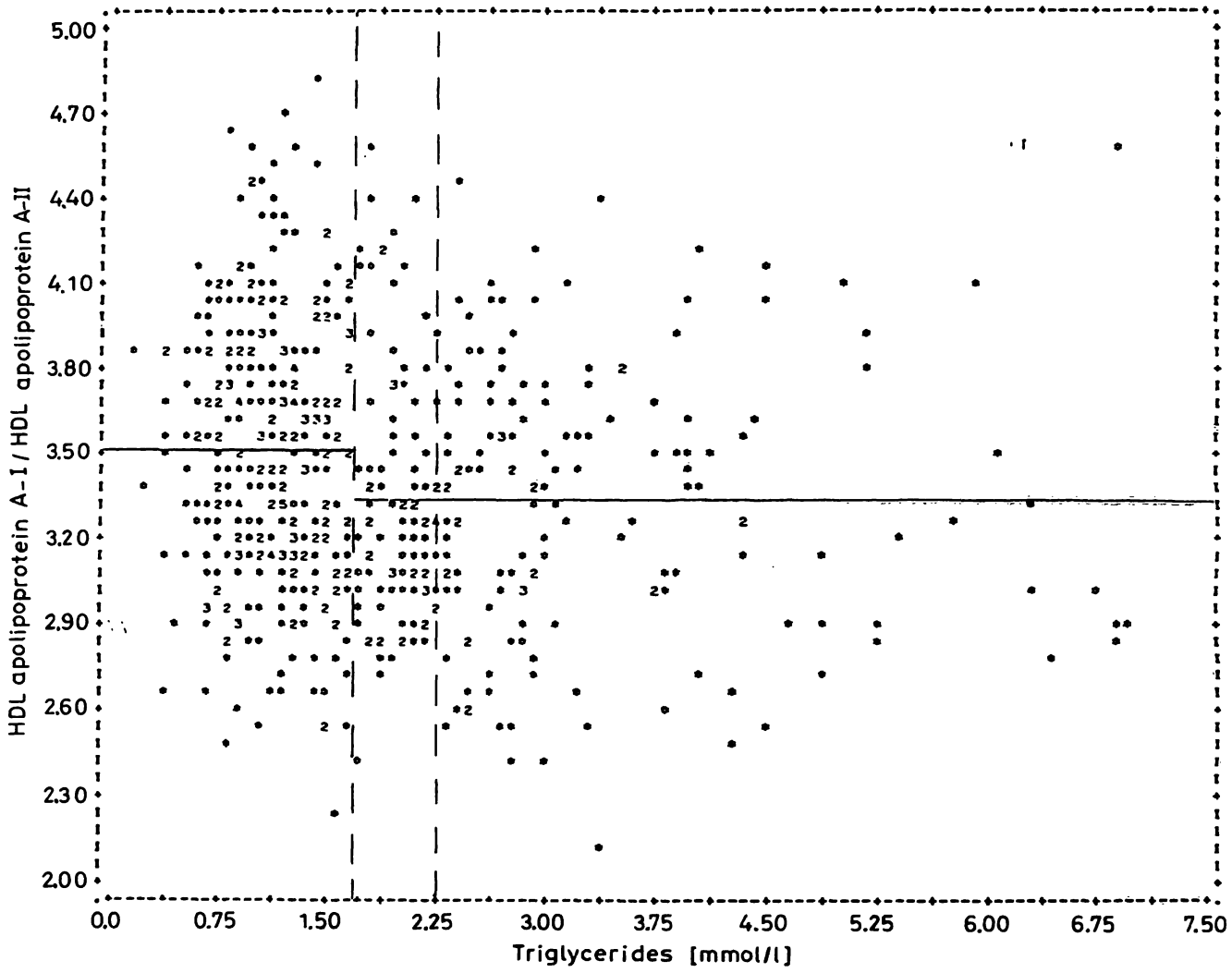

Fig. 7. Bivariate distribution of HDL apolipoprotein A-I/HDL apolipoprotein A-II ratio and triacylglycerol.
r (*Spearman*) = −0.124 ($p < 0.01$)

References

1. Miller, G. J. & Miller, N. E. (1975) Lancet *I*, 16–19
2. Berg, K., Borresen, A. L. & Dahlen, G. (1976) Lancet *I*, 499–501.
3. Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B. & Dawber, T. R. (1977) Amer. J. Med. *62*, 707–714.
4. Yaari, S., Goldbourt, U., Even-Zohar, S. & Neufeld, H. N. (1981) Lancet *I*, 1011–1015.
5. Barboriak, J. J., Anderson, A. J., Rimm, A. A. & King, J. F. (1979) Metabolism *28*, 735–138.
6. Kladetzky, R. G., Assmann, G., Walgenbach, S., Tauchert, P. & Helb, H.-D. (1980) Artery *7*, 191–205.
7. Ishikawa, T., Fidge, N., Thelle, D. S., Forde, O. H. & Miller, N. E. (1978) Eur. J. Clin. Invest. *8*, 179–182.
8. Avogaro, P., Bittolo Bon, G., Cazzalato, G. & Rorai, E. (1980) Atherosclerosis *37*, 69–76.
9. Maciejko, J. J., Holmes, D. R., Kottke, B. A., Zinsmeister, A. R., Dinh, D. M. & Mao, S. J. T. (1983) New Engl. J. Med. *309*, 385–389.
10. Albers, J. J., Cheung, M. C., Hazzard, W. R. (1978) Metabolism *27*, 479–485.
11. Riesen, W. F., Mordasini, R., Salzman, C., Thaler, A. & Gurtner, H. P. (1980) Atherosclerosis *37*, 157–162.
12. Assmann, G., Funke, H. & Schriewer, H. (1982) J. Clin. Chem. Clin. Biochem. *20*, 287–289.
13. Assmann, G., Oberwittler, W., Schulte, H., Schriewer, H., Funke, H., Epping, P. H. & Hauss, W. H. (1980) Internist *21*, 446–459.
14. Assmann, G., Schriewer, H., Schmitz, G. & Hägele, E. O. (1983) Clin. Chem. *29*, 2026–2030.
15. Schriewer, H., Jung, G., Emke, F. & Assmann, G. (1983) J. Clin. Chem. Clin. Biochem. *21*, 611–614.
16. Assmann, G., Schriewer, H. & Funke, H. (1981) J. Clin. Chem. Clin. Biochem. *19*, 273–278.
17. Schriewer, H., Emke, F. & Assmann, G. (1985) J. Clin. Chem. Clin. Biochem. *23*, 355–359.
18. Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. (1972) Clin. Chem. *18*, 499–509.
19. Williams, P., Robinson, D. & Bailey, A. (1979) Lancet *I*, 72–75.
20. Anderson, D. W., Nichols, A. V., Pau, S. S. & Lindgren, F. T. (1978) Atherosclerosis *29*, 161–179.
21. Nichols, A. V. (1967) Human serum lipoproteins and their interrelationships. In: Advances in Biological and Medical Physics (Lawrence, J. H., Gofman, J. W. & Hayes, T. L. eds.) pp. 110–158, Academic Press, New York.
22. Miller, N. E., Hammett, F., Saltissi, S., Rao, S., van Zeller, H., Coltard, J. & Lewis, B. (1981) Br. Med. J. *282*, 1741–1744.

23. Ballantyne, F. C., Clark, R. S., Simpson, H. S. & Ballantyne, D. (1982) Metabolism *31*, 433–437.
24. Eisenberg, S, Gavish, D., Oschry, Y., Fainaru, M. & Deckelbaum, R. J. (1982) J. Clin. Invest. *74*, 470–482.
25. Belfrage, P., Berg, B., Hägerstrand, J., Nilsson-Ehle, P., Tornqvist, H. & Wiebe, T. (1977) Eur. J. Clin. Invest. *7*, 127–131.
26. Hulley, S. B. & Gordon, S. (1981) Circulation *64*, Suppl. 3: III, 57–63.
27. Fraser, G. E., Anderson, J. T., Foster, N., Goldberg, R., Jacobs, D. & Blackburn, H. (1983) Atherosclerosis *46*, 275–286.
28. Barona, E. & Lieber, C. S. (1979) J. Lipid. Res. *20*, 289–315.
29. Sabesin, S. M. (1981) Circulation *64*, suppl. III, 72–84.
30. Haskell, W. L., Camargo, C., Williams, P. T., Vranizan, K. M., Krauss, R. M., Lindgren, F. T. & Wod, P. D. (1984) New Engl. J. Med. *310*, 805–810.
31. Kostner, G. M., Patsch, J. R., Sailer, S., Braunsteiner, H. & Holasek, H. (1974) Eur. J. Biochem. *45*, 611–621.
32. Cheung, M. C. & Albers, J. J. (1977) J. Clin. Invest. *60*, 43–50.

Professor Dr. H. Schriewer
Institut für Klinische Chemie
und Laboratoriumsmedizin
Medizinische Einrichtungen der
Westf. Wilhelms-Universität
Albert-Schweitzer-Straße 33
D-4400 Münster