The Effects of Cigarette Smoking on Serum Levels of HDL Cholesterol and HDL Apolipoprotein A-I

Findings of a prospective epidemiological study on employees of several companies in Westphalia, West Germany

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Summary: In preventive studies of company employees in Westphalia, HDL cholesterol was measured in the sera of 4933 men and 2365 women, as well as HDL apolipoprotein A-I in the sera of 3509 men and 1648 women. Three subgroups were compared:

non-smokers = persons who have never smoked;
ex-smokers = persons who do not smoke at present but did in the past;
smokers   = persons who smoke cigarettes at present.

Mean values for HDL cholesterol and for HDL apolipoprotein A-I were significantly lower in smokers than in non-smokers or in ex-smokers, while there were no differences between the last two groups. These differences appeared in both sexes but were more pronounced in women than in men. To answer the question whether the observed differences are caused by the consumption of cigarettes by itself or whether they are caused by the presence of other risk factors, further subgroups were compared to assess the influence of the risk factors obesity, hypertension, hypercholesterolaemia, hyperglycaemia and hyperuricaemia. It was found that − regardless of the presence of no, one, two or more risk factors − the frequency of probands with low HDL cholesterol values (<0.907 mmol/l (men); <1.166 mmol/l (women)) was about 10% higher in smokers than in non-smokers or ex-smokers. Subgroups based on the number of risk factors did not exhibit the same clear distribution for apolipoprotein A-I values as were seen for HDL cholesterol.

The results are interpreted in the light of the existing literature.

Einfluß des Zigarettenrauchens auf die Konzentration von HDL-Cholesterin und HDL-Apolipoprotein A-I im Blutserum − Ergebnisse der prospektiven epidemiologischen Studie bei Betriebsangehörigen in Westfalen
Zusammenfassung: Im Rahmen präventiver Untersuchungen bei Betriebsangehörigen in Westfalen wurden bei 4933 Männern und 2365 Frauen die HDL-Cholesterinwerte und bei 3509 Männern und 1648 Frauen zusätzlich die HDL-Apolipoprotein A-I-Werte im Blutserum bestimmt. Drei Untergruppen wurden miteinander verglichen:

Nichtraucher = Personen, die niemals geraucht hatten;
Ex-Raucher = Personen, die zur Zeit nicht rauchten, aber früher geraucht hatten;
Raucher   = Personen, die zur Zeit Zigaretten rauchten.

Sowohl die HDL-Cholesterin- als auch die HDL-Apolipoprotein A-I-Werte waren bei Rauchern signifikant niedriger als bei Nichtrauchern oder Ex-Rauchern, während zwischen den beiden letzteren Gruppen kein Unterschied bestand. Die Unterschiede waren bei beiden Geschlechtern vorhanden, bei Frauen jedoch deutlicher als bei Männern ausgeprägt. Zur Beantwortung der Frage, ob die beobachteten Differenzen durch das Zigarettenrauchen selbst oder durch das Vorliegen weiterer Risikofaktoren hervorgerufen wurden, wurden unter Berücksichtigung der Risikofaktoren Übergewicht, Bluthochdruck, Hyperglykämie und Hyperurikämie weitere Untergruppen miteinander verglichen. Es wurde gefunden, daß — unabhängig ob kein, ein oder mehrere Risikofaktoren vorlagen — die Häufigkeit von Probanden mit niedrigen HDL-Cholesterinwerten (<0,907 mmol/l (Männer), <1,166 mmol/l (Frauen)) bei Rauchern etwa 10% höher als bei Nichtrauchern oder Ex-Rauchern lag. In den nach der Anzahl der Risikofaktoren aufgeteilten Untergruppen konnte nicht die gleiche klare Verteilung der Lipoprotein A-I-Werte gezeigt werden wie beim HDL-Cholesterin.

Die Ergebnisse werden unter Berücksichtigung der Literatur diskutiert.

Introduction

Serum HDL cholesterol has been measured increasingly in recent years, due to the fact that there is an apparent negative correlation of HDL cholesterol level and coronary risk (1—4). It has been demonstrated in various epidemiological studies that cigarette smokers have lower HDL cholesterol levels than non-smokers or ex-smokers (5—14). On the other hand, there has been little research to date on the potential effects of other components of HDL (e.g. apolipoprotein A-I, apolipoprotein A-II, phosphatidyl choline, sphingomyelin).

This is a report on the findings of epidemiological studies (15) on the effect of cigarette smoking on HDL cholesterol and HDL apolipoprotein A-I.

Materials and Methods

A total of 4935 male and 2365 female employees of four different companies in Westphalia were examined in the course of our prospective study of company employees. Twelve-hour fasting blood samples were taken in the mornings in a specially equipped bus, and were allowed to stand for 30 minutes at room temperature. Serum was obtained by centrifugation at 3000 min⁻¹, stored at 4 °C and transported to our laboratories within a maximum of three days. The assays were carried out within 24 hours of receiving the samples.

For evaluation purposes, participants in the study were divided into three subgroups, as follows:

1. non-smokers = individuals who had never smoked before;
2. ex-smokers = individuals who did not smoke at the time, but had been smokers in the past;
3. smokers = individuals who smoked cigarettes at the time of the study.

HDL fractions were assayed in the supernatant using the Boehringer Mannheim test (no. 400971) following precipitation of apolipoprotein B-containing lipoproteins by means of phosphotungstic acid/MgCl₂. HDL cholesterol was enzymatically determined using the CHOD-PAP method (Boehringer Mannheim, combination test no. 187313). HDL apolipoprotein A-I was assayed by kinetic nephelometry, as described in detail elsewhere (16).

Results were statistically evaluated using the χ^2 test or the Kruskal-Wallis test, and by multiple comparison of independent samples by the method of Nemenyi or Duncan. The significance level was set at p<0.05.

Results

The effect of cigarette smoking on HDL cholesterol levels

HDL cholesterol values were distributed log-normally in the total group as well as in all considered subgroups.

In smokers the HDL cholesterol level was significantly lower than in non-smokers and ex-smokers although no significant differences were observed between non-smokers and ex-smokers (tab. 1). The disparity was apparent in both sexes, being somewhat more pronounced in women than in men. The proportion of probands with reduced HDL cholesterol (<0.907 mmol/l in men, <1.166 mmol/l in

Tab. 1. Serum HDL cholesterol levels (mmol/l) in company employees.

	Number of Probands	Men Mean Value	Standard Deviation
Non-Smokers	1550	1.167	0.281
Ex-Smokers	1288	1.175	0.291
Smokers	2097	1.126	0.321
Total	4935	1.152	0.302

Comparisons: Non-Smokers/Ex-Smokers, n.s.,
Non-Smokers/Smokers p<0.001,
Ex-Smokers/Smokers p<0.001

	Number of Probands	Women Mean Value	Standard Deviation
Non-Smokers	1346	1.430	0.363
Ex-Smokers	252	1.428	0.348
Smokers	767	1.317	0.315
Total	2365	1.393	0.351

Comparisons: Non-Smokers/Ex-Smokers, n.s.,
Non-Smokers/Smokers p<0.001,
Ex-Smokers/Smokers p<0.001

women) was markedly higher among smokers (by a factor of 1.5) than among the non-smokers or ex-smokers (p < 0.01 at least) (tab. 2).

For the purpose of determining whether the observed differences were solely attributable to cigarette smoking or whether collateral risk factors were involved, probands were divided into three subgroups on the basis of the risk factors listed in table 3. The three groups consisted of probands exhibiting no other risk factor, those with exactly one additional risk factor, and those with two or more additional risk factors. If only the number of risk factors is considered, it is apparent that the differences between non-smokers and ex-smokers on the one hand and smokers on the other hand remain evident in each of the three subgroups (tab. 4). It was markedly apparent among men that the frequency < 0.907 mmol/l was approximately 10% higher in each case among smokers than among non-smokers or ex-smokers, regardless of whether there were additional risk factors involved or not. Among women this difference was evident only when there were no additional or only one additional risk factor present; in cases involving two or more additional risk factors there were no differences observed between female smokers and non-smokers in the group of probands with HDL cholesterol values < 1.166 mmol/l. It

must, however, be taken into account that the number of probands involved was relatively small (22 ex-smokers and 72 smokers).

Tab. 4. Frequency of probands with reduced serum HDL cholesterol (< 0.907 mmol/l in men, < 1.166 mmol/l in women) as a function of cigarette consumption and the number of collateral risk factors.

Total	Men Non-Smokers	Ex-Smokers	Smokers	Total Group
No additional risk factors	8.3% of 855	8.3% of 557	14.2% of 1074	10.9% of 2486
One additional risk factor	19.7% of 441	16.4% of 383	27.3% of 575	21.9% of 1399
Two or more additional risk factors	24.8% of 254	25.0% of 348	36.8% of 448	30.0% of 1050
Total	14.3% of 1550	15.2% of 1288	22.7% of 2097	18.1% of 4935

Total	Women Non-Smokers	Ex-Smokers	Smokers	Total Group
No additional risk factors	14.7% of 748	19.8% of 182	28.9% of 515	20.4% of 1445
One additional risk factor	29.1% of 392	20.8% of 48	40.6% of 180	31.8% of 620
Two or more additional risk factors	37.4% of 206	45.5% of 22	37.5% of 72	38.0% of 300
Total	22.4% of 1346	22.2% of 252	32.5% of 767	25.6% of 2365

Comparisons:

	Men Non-Smokers/ Ex-Smokers	Non-Smokers/ Smokers	Ex-Smokers/ Smokers
No additional risk factors	n.s.	p < 0.001	p < 0.01
One additional risk factor	n.s.	p < 0.05	p < 0.001
Two or more additional risk factors	n.s.	p < 0.01	p < 0.01

	Women		
No additional risk factors	n.s.	p < 0.001	p < 0.05
One additional risk factor	n.s.	p < 0.05	p < 0.05
Two or more additional risk factors	n.s.	n.s.	n.s.

Tab. 2. Frequency of probands with reduced serum HDL cholesterol (< 0.907 mmol/l in men, < 1.166 mmol/l in women).

	Men	Women
Non-Smokers	14.3% (n = 1550)	22.4% (n = 1346)
Ex-Smokers	15.2% (n = 1288)	22.2% (n = 252)
Smokers	22.7% (n = 2097)	32.5% (n = 767)
Total	18.1% (n = 4935)	25.6% (n = 2365)

Comparisons: men: Non-Smokers/Ex-Smokers, n.s.,
                     Non-Smokers/Smokers p < 0.001
                     Ex-Smokers/Smokers p < 0.001
              women: Non-Smokers/Ex-Smokers, n.s.,
                     Non-Smokers/Smokers p < 0.001
                     Ex-Smokers/Smokers p < 0.01

Tab. 3. Defined limit values of risk factors for coronary heart disease.

Obesity	Broca index > = 110%
Hypertension	Blood pressure > = 160/95 mm Hg
Hypercholesterolaemia	Serum cholesterol > = 6.734 mmol/l
Hypertriglyceridaemia	Serum triglycerides > = 2.28 mmol/l
Hyperglycaemia	Serum glucose > = 7.215 mmol/l
Hyperuricaemia	Serum uric acid > = 475.9 µmol/l (men)
	or > = 446.1 µmol/l (women)

In order to determine whether the number of cigarettes smoked every day influences serum levels of HDL cholesterol, the probands were divided into three groups comprising non-smokers, smokers who smoked up to an average of 20 cigarettes a day, and smokers who smoked more than 20 cigarettes a day. Significant differences were apparent in both men and women between non-smokers on the one hand and moderate and heavy smokers on the other, but not between the two groups of smokers (tab. 5). Evaluation of other subgroups divided on the basis of daily cigarette consumption showed that there were no disparities in HDL cholesterol levels between non-smokers and very light smokers (up to 5 cigarettes a day), whereas above 5 cigarettes a day the reduced HDL cholesterol values previously described were again apparent. The degree of serum HDL cholestrol reduction was not influenced by the number of cigarettes smoked per day.

Since the mean values of age were different in non-smokers (36.0 ± 11.5 in men, 37.3 ± 12.6 in women), ex-smokers (41.7 ± 11.4 in men, 33.1 ± 12.6 in women) and smokers (37.3 ± 11.2 in men, 32.0 ± 11.5 in women) and risk factors are highly correlated with age, the computations were repeated for age-tertiles (under 35, 35−45, over 45 years of age) to eliminate the influence of age.

The findings in the age-tertiles do not differ from those in the total group with respect of differences between smokers, non-smokers and ex-smokers but only with respect to the level of mean values obtained. Therefore, these findings are not presented in detail.

The effect of smoking on serum level of apolipoprotein A-1

Apolipoprotein A-I values were also observed to be significantly lowerin smokers than in non-smokers or ex-smokers, though no clear-cut differences were detected between the latter two groups (tab. 6). The differences observed were more marked among women than men. Subgroups based on the number of risk factors present (tab. 3) did not exhibit the same clear distribution for apolipoprotein A-I values as were seen for HDL cholesterol (tab. 7). The female smokers with no additional or only one additional risk factor, and the male smokers exhibiting exactly one risk factor, showed HDL apolipoprotein A-I values lower than those of non-smokers and smokers. No statistical difference was evident, however, between male non-smokers and smokers in the subgroup exhibiting no additional risk factors, or between non-smokers and smokers of both sexes with

Tab. 5. Serum HDL cholesterol levels (mmol/l) ($\bar{x}$ ± S.D.) as a function of cigarette consumption.

		Men	Women
Non-Smokers at present	$\bar{x}$	1.170	1.429
		±0.285	±0.361
	n	2838	1598
1−20 cigarettes per day	$\bar{x}$	1.125	1.319
		±0.309	±0.312
	n	1624	669
>20 cigarettes per day	$\bar{x}$	1.130	1.305
		±0.359	±0.337
	n	473	98

Comparisons:	Men	Women
Non-Smokers/ 1−20 cigarettes per day	$p<0.001$	$p<0.001$
Non-Smokers/ >20 cigarettes per day	$p<0.05$	$p<0.01$
1−20 cigarettes per day/ >20 cigarettes per day	n.s.	n.s.

Tab. 6. Serum apolipoprotein A-I levels (g/l) in company employees.

	Number of Probands	Men Mean Value	Standard Deviation
Non-Smokers	1051	1.370	0.216
Ex-Smokers	866	1.397	0.228
Smokers	1592	1.345	0.233
Total	3509	1.365	0.227

Comparisons: Non-Smokers/Ex-Smokers, n.s.,
Non-Smokers/Smokers p<0.05,
Ex-Smokers/Smokers p<0.001

	Number of Probands	Women Mean Value	Standard Deviation
Non-Smokers	960	1.502	0.246
Ex-Smokers	129	1.494	0.217
Smokers	559	1.425	0.256
Total	1648	1.475	0.250

Comparisons: Non-Smokers/Ex-Smokers, n.s.,
Non-Smokers/Smokers p<0.001,
Ex-Smokers/Smokers p<0.05

two or more additional risk factors. As seen in table 8, there were no apparent differences in HDL apolipoprotein A-I values between moderate (1−20 cigarettes/day) and heavy (>20 cigarettes/day) smokers. Furthermore, no differences were apparent even when smokers were further evaluated on the basis of daily cigarette consumption.

Tab. 7. Mean values and standard deviations of serum apolipo-
protein A-1 levels (g/l) as a function of cigarette con-
sumption and collateral risk factors.

Total		Non-Smokers	Men Ex-Smokers	Smokers	Total Group
No additional	x̄	1.358	1.405	1.346	1.362
risk factors		±0.208	±0.215	±0.288	±0.220
	n	543	346	799	1688
One additional	x̄	1.381	1.384	1.326	1.358
risk factor		±0.223	±0.224	±0.223	±0.225
	n	320	268	442	1030
Two or more	x̄	1.388	1.398	1.367	1.382
additional		±0.227	±0.247	±0.251	±0.244
risk factors	n	188	252	351	791
Total	x̄	1.370	1.397	1.345	1.365
		±0.216	±0.228	±0.233	±0.227
	n	1051	866	1592	3509

Total		Non-Smokers	Women Ex-Smokers	Smokers	Total Group
No additional	x̄	1.505	1.492	1.433	1.476
risk factors		±0.240	±0.229	±0.254	±0.247
	n	482	77	362	921
One additional	x̄	1.487	1.492	1.379	1.456
risk factor		±0.245	±0.177	±0.239	±0.244
	n	317	32	141	490
Two or more	x̄	1.520	1.501	1.488	1.511
additional		±0.520	±0.241	±0.291	±0.269
risk factors	n	161	20	56	237
Total	x̄	1.502	1.494	1.425	1.475
		±0.246	±0.217	±0.256	±0.250
	n	960	129	559	1648

Comparisons:

	Non-Smokers/ Ex-Smokers	Men Non-Smokers/ Smokers	Ex-Smokers/ Smokers
No additional risk factors	p<0.01	n.s.	p<0.001
One additional risk factor	n.s.	p<0.001	p<0.01
Two or more additional risk factors	n.s.	n.s.	n.s.

	Non-Smokers/ Ex-Smokers	Women Non-Smokers/ Smokers	Ex-Smokers/ Smokers
No additional risk factors	n.s.	p<0.001	n.s.
One additional risk factor	n.s.	p<0.001	n.s.
Two or more additional risk factors	n.s.	n.s.	n.s.

Tab. 8. Serum apolipoprotein A-I levels (g/l) (x̄ ± S.D.) as a
function of cigarette consumption.

		Men	Women
Non-Smokers at present	x̄	1.382	1.501
		±0.222	±0.243
	n	1917	1.089
1−20 cigarettes per day	x̄	1.346	1.420
		±0.229	±0.257
	n	1221	485
>20 cigarettes per day	x̄	1.344	1.457
		±0.245	±0.244
	n	371	74

Comparisons:

	Men	Women
Non-Smokers/ 1−20 cigarettes per day	p<0.001	p<0.001
Non-Smokers/ >20 cigarettes per day	p<0.05	n.s.
1−20 cigarettes per day/ >20 cigarettes per day	n.s.	n.s.

Since HDL apolipoprotein A-I values in both sexes
show a correlation with age, the additional influence
of age has been eliminated by repeating the evalua-
tions detailed here for various age groups (under 35,
35−45, and over 45 years of age). The findings do
not indicate any age-related influence on the reduc-
tion in apolipoprotein A-I concentration observed in
conjunction with cigarette smoking.

Discussion

A number of epidemiological studies (8−10,
12−14) indicate that smokers have significantly low-
er levels of HDL cholesterol than non-smokers (dif-
ferences of approx. 0.078−0.130 mmol/l in men,
0.130−0.181 mmol/l in women). Our epidemiologi-
cal findings, conducted on 4935 male and 2365 fe-
male employees of companies in Westphalia, West
Germany, concur with previously published findings.
It has been repeatedly observed that the reduction in
serum HDL cholesterol level is a function of the
number of cigarettes smoked per day (5, 6, 9−14).
However, our study was not able to establish any sig-
nificant differences between the HDL cholesterol
values for moderate (<20/day) and heavy (>20/
day) smokers, among either men or women. The
negative effect of cigarette smoking becomes more
apparent when considered in conjunction with other

factors influencing HDL cholesterol level, such as alcohol consumption (8, 12, 14), use of oral contraceptives (9, 11, 14), or physical exercise (7, 17).

Our own studies show that the differences in HDL cholesterol values between smokers and non-smokers are not attributable to collateral risk factors such as obesity, hypertension, hypercholesterolaemia, hypertriglyceridaemia, hyperglycaemia, and hyperuricaemia. This is an indication that cigarette smoking in itself leads to a reduction in HDL cholesterol level. This assumption is further supported by the observation of other authors (8, 10) as well as our own observations that there are no differences in HDL cholesterol levels between ex-smokers and non-smokers, and that smokers who quit show an increase in HDL cholesterol level in the space of a few weeks (18). It cannot be precluded, however, that this rise in HDL cholesterol level is due to the increase in caloric intake, particularly in the form of fat, which is associated with quitting (18).

Certainly the question of the relevance of the observed effects of cigarette smoking on HDL cholesterol levels is of considerable practical importance. In this regard, it must be borne in mind that the effect of smoking on HDL cholesterol is relatively minor. It must be further taken into consideration that HDL represents a heterogenous group of macro-molecules which differ in particle size, chemical composition, and physicochemical properties. Therefore, it is impossible to determine HDL mass on the basis of HDL cholesterol level and vice versa, particularly in view of the fact that the cholesterol content of HDL varies to between 10 and 20% of the HDL mass. It is in this regard that assaying other components of HDL such as HDL apolipoprotein A-I and HDL phospholipid offers additional valuable information.

It is interesting to note that HDL apolipoprotein A-I values also appear to be lower in smokers than in non-smokers. The differences in HDL apolipoprotein A-I values observed in our study between smokers and non-smokers were, however, in terms of percent, smaller than the differences observed in HDL cholesterol values. This finding is consistent with the results reported by other authors (12, 14, 18). According to studies by Dedonder-Decoopman et al. (12) the drop in apolipoprotein A-I values also appears to be dependent on the number of cigarettes smoked per day. Our studies, however, were not able to establish any significant differences between moderate (<20/day) and heavy (>20/day) smokers.

The epidemiological data presented here leave unanswered the question of the effects of smoking on the metabolism of HDL or any of its components.

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