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Independent association of various smoking characteristics with markers of systemic inflammation in men

Results from a representative sample of the general population (MONICA Augsburg Survey 1994/95)

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KEYWORDS

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Aims Aim of the study was to investigate the association between various markers of systemic inflammation and a detailed history of smoking in a large representative sample of the general population.

Methods and results The effects of chronic smoking on white blood cell (WBC) count, fibrinogen, albumin, plasma viscosity (PV), and high-sensitivity C-reactive protein (CRP) were measured in 2305 men and 2211 women, age 25–74 years, participating in the third MONICA Augsburg survey 1994/95. In men, current smokers showed statistically significantly higher values for WBC count, fibrinogen, PV, and CRP, compared to never smokers, with intermediate, but only slightly increased values for ex-smokers and for occasional smokers. No consistent associations were seen with albumin. Duration of smoking was positively associated with markers of inflammation as were pack-years of smoking. Conversely, duration of abstinence from smoking was inversely related to these markers. Except for WBC count, no such associations were found in women.

Conclusion Data from this large representative population show strong associations between smoking and various markers of systemic inflammation in men. They also show that cessation of smoking is associated with a decreased inflammatory response, which may represent one mechanism responsible for the reduced cardiovascular risk in these subjects.

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Introduction

Cigarette smoking is a powerful risk factor for atherosclerosis. A variety of studies have shown an association of smoking with coronary heart disease (CHD),¹ peripheral artery disease,² aortic aneurysms,³ and stroke.⁴ On the other hand, after smoking cessation a time-dependent decrease in the risk of acute myocardial infarction and stroke, to a level almost similar to persons who never smoked, has been reported.⁵ In general, the full risk reduction was seen within 5–10 years after abstinence from smoking.

Mechanisms by which cigarette smoke may initiate or accelerate atherosclerosis and its complications are not fully understood. Smoking causes coagulation and lipid abnormalities,⁶ and circulatory effects due to enhanced catecholamine release have been reported.⁷ Recent work focuses on smoking-induced endothelial injury, and there is evidence that mainly free radicals, such as nitric oxide radicals, singlet oxygen and hydrogen peroxide, mediate cigarette-smoke induced endothelial damage.⁸ Oxidative stress promotes a systemic acute phase response possibly by NF- κ B activation⁹ and cytokine induction,¹⁰ and an association of cigarette smoking with a variety of acute phase markers such as inflammatory cytokines,^{11,12} cell adhesion molecules,^{12,13} C-reactive protein (CRP),^{14–23} fibrinogen,^{18,24–27} plasma viscosity (PV),^{24,28–30} white blood cell (WBC)-count^{6,31–33} and albumin^{16,34,35} has been reported. Therefore, markers of inflammation may directly indicate smoking-induced inflammatory responses. Additionally, the association of these markers of inflammation with smoking may modulate the smoking-associated risk, since several of them are by itself strong and independent risk factors for CHD.³⁶

Some of the above mentioned studies merely investigated the association of solitary inflammatory markers with smoking status. Only incomplete data are available taking into account other smoking characteristics, such as duration of smoking, smoked pack-years, and duration of abstinence from smoking. Thus, in the present large population-based study we aimed to systematically investigate the association of the smoking status (never, ex, occasional, regular) with a variety of markers of systemic inflammation (CRP, fibrinogen, PV, WBC-count, albumin), and also to assess the association of various smoking characteristics with these markers.

Methods

The MONICA Project was a WHO-coordinated observational long-term study with the main aims of

Table 1 Baseline characteristics and markers of inflammation

Variable	Men	Women
Age (years)	49.9 (14.2)	49.0 (13.8)
BMI (kg/m ²)	27.4 (3.7)	26.6 (5.1)
Total cholesterol (mmol/l)	6.02 (1.17)	5.95 (1.14)
HDL-cholesterol (mmol/l)	1.24 (0.37)	1.56 (0.43)
Systolic blood pressure (mmHg)	135 (18.0)	129 (20.4)
Diastolic blood pressure (mmHg)	82 (11.4)	78 (11.0)
Alcohol consumption (g/d) ^a	25.7 (2.9–40)	7.5 (0–11.4)
Diabetes n (%)	120 (5.0)	103 (4.2)
Markers of inflammation		
CRP (mg/l) ^b	1.33 (3.05)	1.43 (3.26)
Fibrinogen (g/l) ^b	2.90 (0.71)	2.92 (0.71)
Plasma viscosity (mPa·s) ^b	1.229 (0.066)	1.222 (0.063)
WBC count (10 ³ /μl) ^b	7.2 (1.9)	7.1 (1.8)
Albumin (g/l)	44.3 (4.7)	42.4 (4.4)

^aMedian (interquartile range)

^bAntilogs of ln-transformed data. Data expressed as mean (standard deviation), or percentages.

Table 2 Smoking status, relative frequencies (%)

Sex	Age (years)	n	Never	Ex	Occasional	Regular
Men	25–34	435	36.8	21.4	4.8	37.0
	35–44	438	31.3	29.2	3.2	36.3
	45–54	471	31.2	37.4	4.7	26.8
	55–64	496	35.9	42.7	1.6	19.8
	65–74	465	27.1	57.0	1.9	14.0
Women	25–34	414	47.6	19.6	4.1	28.7
	35–44	475	41.9	27.4	3.6	27.2
	45–54	478	57.7	21.3	3.1	17.8
	55–64	471	76.4	13.0	1.1	9.6
	65–74	373	75.9	15.0	1.1	8.0

measuring trends in cardiovascular morbidity and mortality and assessing their relationship to changes in risk factor levels and medical care.^{37,38} It comprised a coronary event register and three independent cross-sectional studies in 5-year intervals. The present analysis involved all 2305 men and 2211 women aged 25–74 years among those participating in the third MONICA Augsburg survey in 1994/95. The participation rate was 74.9%. Smoking characteristics such as smoking status (never smoker, ex-smoker, occasional smoker, regular smoker), type of smoked tobacco product (cigarettes, cigars, cigarillos, pipe), duration of smoking (years), number of smoked cigarettes, and duration of abstinence from smoking (years), were recorded in detail by a standardized questionnaire according

Table 3 Distribution of smoking characteristics in the adult general population^a

Sex	Age (years)	Cigarettes per day (n)	Pack-years	Duration of smoking (years)	Abstinence from smoking (years)
Men	25–34	18 (0–50)	8.40 (0–40.00)	11 (2–20)	5 (0–16)
	35–44	20 (0–60)	15.00 (0–56.00)	20 (2–28)	9 (0–24)
	45–54	20 (0–60)	24.00 (0–76.00)	27 (2–39)	16 (0–34)
	55–64	20 (0–60)	21.00 (0–85.00)	31 (2–47)	20 (1–39)
	65–74	10 (0–40)	16.50 (0–84.00)	32 (2–55)	23 (1–51)
Women	25–34	10 (0–35)	5.50 (0–29.75)	11 (2–20)	5 (0–14)
	35–44	15 (0–40)	10.45 (0–37.5)	19 (2–27)	12 (0–24)
	45–54	10 (0–40)	8.93 (0–48.00)	26 (0–36)	15 (0–31)
	55–64	8 (0–40)	9.30 (0–58.80)	29 (2–45)	17 (0–31)
	65–74	8 (0–25)	10.50 (0–59.80)	33 (1–54)	16 (0–47)

^aData expressed as median (IQ-range)

to the recommendations of the WHO.³⁹ Cigarette pack-years were computed as duration of smoking (years) multiplied by the number of smoked cigarettes, divided by 20. Blood pressure was measured by a Hawksley random zero sphygmomanometer. Body height and weight were measured in light clothing, and BMI was computed as weight divided by the square of height (kg/m²). Alcohol consumption was determined as described elsewhere.⁴⁰

Non-fasting venous blood samples were drawn into EDTA with the subjects in a supine resting position with only short term venous occlusion and minimal suction. Red blood cell count, WBC count, haematocrit and platelet count were determined (Coulter Counter). EDTA blood was immediately centrifuged at 3000 g for 15 min, and the plasma aliquoted and stored at –70 °C. PV was measured at 37 °C in a Coulter-Harkness capillary viscometer (Coulter Electronics, Luton, UK).⁴¹ Fibrinogen and albumin concentrations were determined by immunonephelometry (Dade Behring, Marburg, Germany). CRP concentration was measured by an immunoradiometric assay (range 0.05–10 mg/l) calibrated with the WHO reference standard 85/506.⁴² Total and HDL-cholesterol concentrations were measured by routine enzymatic methods (CHOD-PAP, Boehringer, Mannheim, Germany), and LDL cholesterol was calculated according to Friedewald.⁴³

Routine laboratory tests, including blood cell counts, total cholesterol and HDL-cholesterol were subject to continuous internal and external quality control. Coefficients of variation for repeated measurements were 0.7% for PV, 5.0% for fibrinogen, 6.9% for albumin and 12% for CRP over all ranges.

Statistical analysis

Baseline characteristics and markers of inflammation of the study population are described as means

and standard deviations, if not indicated otherwise. Values have been calculated separately for men and women. CRP, fibrinogen, PV, and WBC count values were transformed to natural logarithms for greater symmetry of the distribution. Numbers shown in the tables are back-transformed to the original scale by taking antilogs, thus representing geometric means. The following analyses were restricted to cigarette smokers because of the small numbers of cigar, cigarillo and pipe smokers. Relative frequencies of the smoking status were calculated separately for men and women and for different age groups. Medians and interquartile ranges of different smoking characteristics were calculated for the same categories. Adjusted means of different markers of inflammation (CRP, fibrinogen, PV, WBC count, albumin) were computed according to the smoking status (never, ex, occasional, regular smoke exposure) and in quintiles of different smoking characteristics (number of smoked cigarettes, duration of smoking, pack-years, duration of abstinence from smoking). Adjustment was performed for multiple possible confounding covariates such as BMI, total cholesterol, HDL-cholesterol, alcohol consumption, systolic and diastolic blood pressure, education, physical activity, diabetes, CHD and creatinine. The *p*-values represent probability values for testing the simultaneous equality of the means. All computations were performed with SAS software (SAS Windows 6.12). *P*-values <0.05 were considered statistically significant.

Results

Baseline characteristics of the study population and markers of inflammation are reported in Table 1 for men and women separately. Men and women differ in their lipid profile with higher total cholesterol

Table 4 Smoking characteristics and markers of systemic inflammation

Sex	Smoking characteristics	CRP (mg/l)	Fibrinogen (g/l)	PV (mPa-s)	Albumin (g/l)	WBC-count ($10^3/\mu\text{l}$)
Smoking status						
Men	Never	1.03	2.71	1.216	44.5	6.5
	Ex	1.27	2.78	1.220	44.6	6.7
	Occasional	1.41	2.88	1.227	43.8	6.7
	Regular	1.92	2.99	1.226	43.9	7.9
	<i>P</i> value ^a	<0.001	<0.001	0.008	0.031	<0.001
Women	Never	1.41	2.83	1.217	42.5	6.7
	Ex	1.39	2.74	1.209	42.6	6.6
	Occasional	1.15	2.79	1.215	42.1	7.1
	Regular	1.52	2.94	1.213	42.5	7.8
	<i>P</i> value ^a	0.254	<0.001	0.038	0.864	<0.001
Cigarettes per day						
Men	0–3	1.35	2.80	1.220	44.3	6.9
	4–12	1.48	2.85	1.221	44.3	7.1
	13–20	1.71	2.92	1.227	44.4	7.4
	21–30	1.63	2.94	1.228	43.6	7.6
	35–100	1.63	2.92	1.230	44.3	7.2
	<i>P</i> value ^a	0.021	0.023	0.118	0.296	<0.001
Women	0–0	1.24	2.71	1.205	42.1	6.7
	1–8	1.26	2.69	1.202	42.7	6.9
	9–15	1.29	2.70	1.202	42.9	7.5
	16–20	1.24	2.72	1.206	43.1	7.4
	21–60	1.50	2.72	1.208	42.4	7.6
	<i>P</i> value ^a	0.689	0.288	0.721	0.226	<0.001
Cigarette pack-years						
Men	0.0–1.3	1.30	2.79	1.219	44.3	6.8
	1.4–1.0	1.43	2.79	1.223	44.7	6.9
	11.1–21.0	1.63	2.91	1.223	44.4	7.1
	21.3–36.0	1.71	2.98	1.228	43.8	7.6
	36.1–176.4	1.79	2.96	1.234	44.1	7.6
	<i>P</i> value ^a	0.001	<0.001	0.027	0.150	<0.001
Women	0.0–0.0	1.23	2.71	1.205	42.2	6.6
	0.2–5.0	1.35	2.67	1.202	43.1	6.9
	5.2–12.0	1.21	2.69	1.203	43.2	7.1
	12.1–23.0	1.10	2.76	1.203	42.7	7.8
	23.5–96.0	1.57	2.77	1.206	42.2	7.7
	<i>P</i> value ^a	0.045	0.357	0.969	0.115	<0.001
Duration of smoking (years)						
Men	0–11	1.24	2.78	1.220	44.6	6.7
	12–18	1.40	2.78	1.220	44.5	6.8
	19–25	1.48	2.86	1.223	44.0	7.1
	26–35	1.83	3.00	1.228	44.2	7.6
	36–60	2.02	3.03	1.235	43.9	7.9
	<i>P</i> value ^a	<0.001	<0.001	0.029	0.364	<0.001
Women	0–9	1.30	2.67	1.206	42.9	6.6
	10–14	1.20	2.63	1.200	43.1	6.9
	15–21	1.29	2.75	1.202	42.5	7.4
	22–29	1.22	2.74	1.199	42.7	7.6
	30–56	1.41	2.82	1.215	42.1	7.6
	<i>P</i> value ^a	0.761	0.047	0.058	0.370	<0.001
Abstinence from smoking (years)						
Men	0–6	1.77	2.90	1.232	44.0	6.9
	7–12	1.53	2.92	1.227	44.3	6.7
	13–20	1.43	2.88	1.229	43.7	6.8
	21–29	1.21	2.82	1.218	44.5	6.4
	30–55	1.25	2.81	1.217	44.1	6.7
	<i>P</i> value ^a	0.014	0.485	0.087	0.440	0.044

Table 4 (continued)

Sex	Smoking characteristics	CRP (mg/l)	Fibrinogen (g/l)	PV (mPa·s)	Albumin (g/l)	WBC-count ($10^3/\mu\text{l}$)
Women	0–3	1.14	2.58	1.196	41.8	6.7
	4–8	1.41	2.68	1.203	42.7	6.8
	9–14	1.36	2.70	1.209	42.8	7.0
	15–20	1.44	2.69	1.208	43.0	6.5
	21–50	1.32	2.74	1.201	42.7	6.3
	<i>P</i> value ^a	0.671	0.486	0.468	0.503	0.122

^a*P* values for testing the equality of the means. Means (adjusted for age, BMI, total cholesterol, HDL-cholesterol, alcohol consumption, systolic and diastolic blood pressure, education, physical activity, diabetes, CHD and creatinine) computed from normalized transformed values.

and lower HDL-cholesterol concentrations in men, and a substantially higher alcohol consumption in men. On the other side, women tend to have higher concentrations of CRP than men.

Smoking is more prevalent in men than in women (Table 2). In general, regular smoking is more common in younger individuals than in the older above age 55 years. There are also a large number of ex-smokers in this age group, particularly among men. Men are heavier smokers than women (Table 3), but there are no differences in smoking duration. The age at onset of smoking (derived from the duration of smoking) tends to be the same in both sexes, with a tendency to an earlier beginning (in the teenage years) in younger individuals.

The associations of smoking status and different smoking characteristics with markers of systemic inflammation are shown in Table 4. All means presented have been adjusted for a variety of potential confounders by multiple linear regression. In men, the positive acute phase markers CRP, fibrinogen, PV and WBC count are positively associated with smoking status, with highest plasma concentrations in regular smokers, slightly increased concentrations in occasional and ex-smokers, and lowest concentrations in never smokers. Comparing never smoking with regular smoking men, plasma concentrations of CRP are nearly doubled in regular smokers (1.03 mg/l vs 1.92 mg/l). In men, the number of smoked cigarettes and pack-years and duration of smoking are positively, whereas duration of abstinence from smoking is inversely associated with these markers of systemic inflammation. This result is very consistent and statistically significant for all positive acute phase markers, except for the inverse association of fibrinogen and PV with the duration of abstinence from smoking and PV with the number of smoked cigarettes per day. There is no association of albumin – an unspecific negative acute phase marker – with parameters of smoking. Furthermore, except for WBC count, there is no

association of smoking with these markers of inflammation in women.

Discussion

In this large, population-based study we found a consistent and statistically highly significant association of positive acute phase markers with smoking in men. On the other hand, acute phase markers were negatively associated with years since cessation of smoking. Except for WBC count no such associations were found in women.

Several previous studies have reported an association of smoking with CRP,^{14–23} but only few analysed in detail the association of smoking characteristics, such as the number of smoked cigarettes,¹⁴ pack-years^{17,19} and abstinence from smoking,^{15,17,23} with CRP in a large representative population-based sample. Comparing current smokers with never-smokers, the magnitude of the difference seems to be higher in men (e.g. ~1.5 mg/l in the Physicians Health Study¹⁴), compared to women (e.g. 0.08 mg/l in the Women's Health Study¹²). This is in accordance with the missing difference in women reported here. All of the previously reported data were cross-sectional, except one.¹⁵ In the course of this retrospective study, a non-significant decrease in CRP concentration (–0.21 mg/l) after smoking cessation of one year was reported. Conversely, in the present study reported smoking cessation covers a longer period (up to >30 years), and there was a greater difference in CRP concentration in men of 0.67 mg/l, comparing smoking abstainers in the highest quintile (30–55 years of abstinence) to current smokers.

Significantly higher fibrinogen concentrations in smokers^{24,26,27} with a dose-response relationship have been shown by others.²⁵ In some reports PV values^{24,28,29} parallel the changes in fibrinogen concentrations, as we also could show in the present analyses. This is not surprising, since fibrinogen is a

major determinant of PV.⁴⁴ Again, smoking cessation was not associated with lower PV and fibrinogen concentrations in women, and this corresponds with data from a longitudinal study²⁸ in which men experienced a significant decrease in PV of -0.021 mPa·s after 2 years of smoking cessation, and women did not (difference -0.009 mPa·s, ns).

In the present study, WBC count represented the parameter most reliably associated with smoking in both men and women. The association of WBC count with smoking has been reported previously^{6,31–33}, showing a dose-response relationship^{31,32} and an association with smoking duration.³² Overall, the magnitude of the effect of smoking on WBC count (difference $1.5–2 \times 10^3/\mu\text{l}$) is comparable throughout the studies.

In contrast to other studies^{34,35} we found an inverse association of the negative acute phase marker albumin with smoking in men only. Both, Das et al.¹⁶ and Dales et al.³⁴ revealed significantly lower albumin levels in smokers, compared to non-smokers, but once more the difference was more prominent among men (men: 7.9 g/l, women: 4 g/l¹⁶). A dose-response relationship and an association of albumin with abstinence from smoking was not observed in the present study, nor was it reported by others. The relatively weak association of albumin with smoking may be due to the fact that besides inflammation, serum albumin concentrations are mainly determined by other conditions such as nutritional status and liver function.

There are several possible mechanisms by which smoking may be associated with systemic markers of inflammation. First, acute phase proteins may be surrogates for smoking-induced chronic inflammatory processes such as periodontitis,⁴⁵ airway inflammation⁴⁶ and atherosclerosis itself.⁴⁷ Second, certain compounds of smoke such as free radicals¹¹ and phenol-rich glycoproteins⁴⁸ directly exert an inflammatory stimulus on macrophages which may trigger the production of inflammatory cytokines such as $\text{TNF}\alpha$, IL-1, and IL-6.¹¹ And third, it is conceivable that there is an indirect effect by nicotine-induced catecholamine release⁷ which modulates the systemic and local cytokine balance.⁴⁹

Although women are more sensitive to harmful effects of smoking,¹ in the present study except for WBC no association of smoking and smoking characteristics with markers of the acute phase response were observed in women, compared to men. Taking the above mentioned mechanisms into consideration, one may speculate that women experience less smoking-related inflammatory reactions, e.g. airway inflammation. This may be attributable

to a different puffing behaviour in women. Generally, women use less untipped and more low-yield cigarettes,⁵⁰ and take smaller and shorter puffs.⁵¹ However, the sex differences in self-reported inhaling habits disappear in pharmacological motivated heavy-smoking women (>20 cigarettes/d).⁵⁰ This is in line with our observation of markedly elevated inflammatory parameters just in the 5th quintile (>21 cigarettes/d) of cigarette consumption in women.

The major strength of the present analysis is the fact that data are based on a large representative sample (participation rate 74.9%) of the general population including both sexes and a large age range. However, the results may be limited due to the cross-sectional nature of the study which excludes establishing causal relationships. Furthermore, residual confounding for example by other lipid variables and homocysteine may represent a problem. However, this does not seem to be critical since age-adjusted values were not significantly altered in multivariate analyses. Finally, data may be biased by incorrect reporting of smoking habits, mainly because we did not perform objective measures of cigarette consumption, such as cotinine plasma concentrations.

In summary, we found a strong and consistent association of markers of systemic inflammation with smoking and various smoking characteristics, particularly in men. The low grade systemic acute phase response may contribute to the increased cardiovascular risk in smokers. Markers of systemic inflammation are inversely associated with years since cessation of smoking. Thus, our data provide further evidence that immediate smoking cessation should be strongly recommended in particular to those individuals with CHD. Different smoking patterns may explain the less consistent results in women.

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