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Blood Rheology in Healthy Cigarette Smokers
Results from the MONICA Project, Augsburg

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To investigate the relationship between cardiovascular risk factors and the fluidity of blood, a random sample of the population consisting of 4022 persons ages 25 to 64 years was investigated for plasma viscosity, hemoglobin, and total serum protein. A total of 567 healthy nonsmokers and 287 healthy smokers were identified and compared. Plasma viscosity was found to be elevated in male smokers; this was related to both the degree and the duration of cigarette abuse. Plasma viscosity proved to be age-dependent in smokers, but did not change with age in nonsmokers. Total serum protein decreased with age in nonsmokers, while it did not change in smokers. Hemoglobin increased only in female smokers. These findings suggest that blood fluidity is jeopardized in smokers. In men the hemorheological deficit is mostly due to a rise of plasma viscosity, which, in turn, may be caused by an elevation of plasma fibrinogen levels. In women it is predominantly due to an increase in hemoglobin. These alterations in hemorheological variables may be a marker for increased cardiovascular risk in smokers and could reduce blood flow and hinder microcirculatory function. (Arteriosclerosis 8:385-388, July/August 1988)

Chronic cigarette smoking is one of the most prominent cardiovascular risk factors. Smoking can lead to a reduction in blood flow in most organs via a variety of mechanisms. The fluidity of blood, which is determined by plasma viscosity and hemoglobin levels, is also closely related to cardiovascular risk and may influence volume flow. Therefore, the hemorheological effects of chronic smoking are of interest in arteriosclerosis research. It has been suggested that both acute and chronic smoking affect blood rheology. Studies on the effects of chronic smoking have unfortunately been hampered by the fact that the populations investigated were burdened by concomitant diseases or risk factors. The present study evaluates the effects of chronic cigarette smoking on hemorheological variables in otherwise healthy people identified from a large random sample of the population.

Subjects
Participants were interviewed by trained personnel using a standardized questionnaire. The questionnaire concerned the participant's medical history, attitude toward and knowledge of health, use of medical care, life style, socioeconomic variables, and drug history for the 7 days preceding the examination. If a participant was identified as an actual smoker ("Do you presently smoke cigarettes"?), he or she was then asked, "How old were you when you started smoking?" The intensity of smoking behavior was assessed by the question, "How many cigarettes do you smoke, on average, per day?" For the purpose of the present analysis, it was assumed that the number of cigarettes smoked per day had been constant in the past.

No participant selected for the present analysis had a history of cardiovascular or other chronic diseases, showed an abnormal electrocardiogram (ECG), or took medication known to influence blood rheology. Systolic and diastolic blood pressures were less than 160 and 95 mm Hg respectively, total cholesterol was below 260 mg/dl (6.72 mol/l), uric acid was less than 400 moli/l (men) and 340 moli/l (women), and body mass index (BMI = weight [kg]/height [m]^2) was below 30. There were 567 nonsmokers and 287 smokers in the study population who met these criteria. Exsmokers were allocated to the non-smoker group. Informed consent was obtained from all participants.

Methods

Study Design
The MONICA project, Augsburg is part of an epidemiological study coordinated by the World Health Organization with 40 centers in 27 countries. Its main objective is the evaluation of trends in cardiovascular morbidity, mortality, and case fatality over 10 years in defined populations (register studies). Furthermore, this project investigates the determinants of cardiovascular diseases by multiple cross-sectional studies (population survey). For the first survey (conducted between October, 1984 and May, 1985), a total of 5312 men and women ages 25 to 64 years from a target population of 282,779 inhabitants were studied. Two-stage age-sex-stratified cluster sampling was applied. A response rate of 79.4% was achieved (4022 persons from 5069 available).
Blood Sampling

Blood samples were drawn from approximately 90% of the persons studied. The sampling technique was in accordance with the recommendations of the International Committee for Standardization in Haematology. Blood was drawn by venipuncture with a 19G canula after the volunteers had been sitting for at least 30 minutes. Only short-term venous occlusion and minimal suction were applied. In a subsample of 1047 persons, the venous occlusion time was recorded and related to the hemorheological variables. No significant correlations were found. Part of the venous blood was left to clot for serum samples; part was anticoagulated with 1.5 mg EDTA per ml.

Procedures

Whole EDTA blood was taken to measure hemoglobin by the cyanate method (TOA hemoglobin meter and diluter) and was subsequently centrifuged at 3000 g for 15 minutes. A Harkness-Coulter capillary viscometer (Coulter Electronics, England), adjusted to 37°C, was used to measure plasma viscosity. The measurement procedure and sample preparation met the criteria of the International Committee for Standardization in Haematology. Total serum protein (g/dl) measurements were performed in duplicate and plasma viscosity (mPa-s) tests in triplicate. For quality control, hemoglobin and plasma viscosity measurements were compared to standard solutions. At irregular intervals, duplicate samples were measured in a single-blind fashion. There was no baseline shift during the 8 months’ trial period. The intra-assay variation coefficient for plasma viscosity in our laboratory has been shown to be 0.3% with an intra-individual variation of 1.3% on 7 days.

Blood pressure (Hawksley Random Zero Sphygmomanometer), body height and weight, total cholesterol, high density lipoprotein (HDL) lipoprotein, apolipoprotein, gamma-GT, uric acid, creatinine levels, and a 12-lead ECG were determined for participants by standard techniques.

Statistical Methods

To examine the relationship between smoking (independent variable) and hemoglobin, plasma viscosity, and total serum protein (dependent variables) and their possible relation to age, a separate linear model was applied for men and women. A linear regression analysis with dummy variables was chosen. Since numbers in the higher age categories were small, analyses were run with only three age groups (25 to 34, 35 to 44, and 45 years and older). Interactions between age and smoking were considered. To evaluate the effect of the duration of smoking on hemorheological parameters, duration was used as a dichotomous variable (<20 vs ≥20 years). The same applied to the number of cigarettes per day (<20 vs ≥20) and to age (<45 vs ≥45 years). Calculations were done using the GLM program of the SAS statistical package.

Results

Table 1 and Table 2 show the mean values and standard errors of the mean (SEM) for hemoglobin, plasma viscosity, and total serum protein according to age for healthy nonsmokers and healthy smokers.

Effect of Smoking on Hemoglobin

The effect of smoking was not modified by age in men. There was no significant difference (p>0.1) in hemoglobin between smokers and nonsmokers. In female smokers, hemoglobin was significantly higher compared to female nonsmokers (13.7 vs. 13.3 g/dl, p<0.02).

Effect of Smoking on Plasma Viscosity

For men there was a consistent relationship between age and plasma viscosity within smokers and a lack of one within nonsmokers (interaction with p=0.002). For male smokers 45 years old or older were
associated with significantly elevated plasma viscosity values compared to nonsmokers. In women, the effect of smoking was not modified by age (interaction with $p=0.20$; Figure 1B). There was, however, a nonsignificant trend for plasma viscosity to increase in female smokers with age, similar to the one found in men. The mean values of plasma viscosity were not significantly different between smokers and nonsmokers (1.23 vs. 1.22 mPa-s; $p=0.29$). No significant effect of age ($p=0.41$) was noted.

**Effect of Smoking on Total Serum Protein**

For men there was a significant interaction between age and smoking ($p=0.03$). Total serum protein declined with age in nonsmoking men and was nearly constant in smokers. In smokers, the values of serum protein for the two lower age groups paralleled those of nonsmokers on a slightly lower level (Figure 2A). In women, age ($p<0.02$) and smoking ($p<0.03$) were significantly related to total serum protein. Total serum protein in female nonsmokers was higher than in smokers (71.4 vs. 70.7 g/l) (Tables 1 and 2). As with men, female nonsmokers showed a decrease of total serum protein with age, while smokers did not (Figure 2B). The interaction was weaker than with men ($p=0.06$).

**Effect of Duration of Smoking on Plasma Viscosity**

Plasma viscosity in men showed a strong dependence on smoking habits modified by age. Because of the interaction between smoking and age, which was found mainly in the higher age group, the variables, "duration of smoking" and "number of cigarettes," were included in a model for smokers. This analysis was restricted to plasma viscosity in men. A weak interaction between the duration of smoking and the number of cigarettes smoked was found ($p=0.07$). Heavy smokers who had smoked for 20 years or longer were associated with higher plasma viscosity than were smokers with a shorter smoking history (Figure 3). However, the number of cigarettes smoked per day had a statistically significant main effect on plasma viscosity in male smokers ($p=0.03$). The effect of age was also significant ($p=0.02$).

**Discussion**

Plasma viscosity increased only in male smokers, while hemoglobin was elevated only in female smokers. Higher hemoglobin levels have been reported in smokers of both sexes. The discrepancy between these results and our findings in men might be due to selection criteria: in most studies, smoking is associated with other cardiovascular risk factors or diseases. Our study used strict selection criteria for health, excluded concomitant risk factors, and suggests that hemoglobin in men changes only marginally as a function of smoking habits in the absence of other risk factors or disease.

Plasma viscosity is raised in male chronic smokers. Theoretically, this could be due either to a contraction of plasma volume or to an increase in synthesis of macromolecules, which exert a strong influence on plasma viscosity. In a smaller study with only male volunteers,
the fibrinogen and plasma viscosity increase in male smokers was shown to be related to cigarette consumption. Furthermore, these changes were reversible within 3 months after cessation of smoking. If the fibrinogen effect were long lasting, as suggested recently, oxsmokers (who are included in the nonsmoker group) might have elevated fibrinogen levels. This would minimize the difference in plasma viscosity as seen in this study. Our results suggest that increased macroprotein levels are the predominant cause for elevation in plasma viscosity in men, because plasma volume contraction would, of course, also cause an increase in hemoglobin and total serum protein.

The positive correlation between plasma viscosity and age in male smokers may be explained by a weak interaction of duration of smoking and intensity of smoking behavior. Figure 1B seems to imply that plasma viscosity in female smokers is also age-dependent. This effect, however, failed to reach the level of statistical significance. The duration of smoking history and the number of cigarettes smoked affected the plasma viscosity in men (Figure 3). Interestingly, the duration of smoking also represents an indicator of risk for ischemic heart disease. In contrast, hemoglobin was independent of age, confirming other trial results.

At present it is not known why women yielded different results than men. In women the threshold for increased fibrinogen synthesis could be higher than in men. If this is the case, only excessive smoking would lead to a rise in plasma viscosity, while the results from the total female population would be largely unchanged. In our analysis, female smokers showed an increase in hemoglobin, but their plasma viscosity values were unaffected by smoking. This suggests an enhanced erythropoiesis in female smokers. Hence, the threshold for fibrinogen synthesis in women may be higher and the threshold for an erythropoietic stimulus may be lower.

The biological meaning of these hematological alterations may be twofold. First, they could be markers of an increase in arteriosclerotic risk. According to our working hypothesis, both the early endothelial damage and the changes leading to decreased blood fluidity are caused by similar surface adsorption phenomena. This could explain the striking association between cardiovascular risk factors and pathological flow properties of blood. Second, the viscosity of blood will increase the viscous component of the peripheral resistance and could participate in reducing blood flow in organs such as the heart or the brain. With all other factors constant, perfusion is directly dependent on the fluidity of blood. In situations where the vasoconstrictor reserve is limited, hemorheological mechanisms can lead to a substantial reduction in volume flow. On the microcirculatory level, rheological properties of blood cells may interfere with vasomotion, leading to a significant maldistribution of oxygen supply. Both these possibilities need further, careful evaluation and experimental testing.

References