Acute changes in atherogenic and thrombogenic factors with cessation of smoking

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Summary
Tobacco smoking is associated with alterations in several factors considered to be important in the atherosclerotic process. Thirty chronic smokers were studied 2 weeks before and 2 weeks after complete tobacco withdrawal. Significant reductions in fibrinogen, haematocrit, plasma viscosity and whole blood viscosity as well as a significant increase in HDL-cholesterol were observed. As these factors are important in both atherogenesis and thrombogenesis, these observations may give insight into tobacco-induced atherosclerotic disease and may be responsible for the more rapid reduction in the incidence of cardiovascular disease that is believed to occur after stopped smoking.

Introduction
Tobacco smoking is a well described independent risk factor for several cardiovascular events including heart attack and stroke. Epidemiological studies have shown the strong synergistic effects of smoking with other risk factors such as hypertension and hypercholesterolaemia on cardiovascular disease[1,2]. Recently, several studies have shown that smokers who abstain after myocardial infarction have a significant reduction in mortality over several years compared with those who continue to smoke[3-8]. Atherosclerotic disease is a consequence of atherogenic and thrombogenic mechanisms[9-12] and smokers often have identifiable abnormalities in both atherogenic and thrombogenic factors[13-20]. However, little is known about the timescale over which withdrawal of tobacco affects these atherogenic and thrombogenic factors. The aim of the present study was to assess, in a group of chronic smokers, the acute effects of complete tobacco withdrawal on these factors and possibly to highlight some of the tobacco related mechanisms associated with cardiovascular disease.

Patients and methods
Thirty (12 males and 18 females) chronic smokers of more than 5 years duration who smoked between 7 and 53 (median 20) cigarettes per day were studied while attending a smoking cessation course. Median age was 38 (range 20-68) years. Subjects were studied 2 weeks prior to and 2 weeks after stopping smoking. At each visit subjects completed a 24-h dietary recall and exercise questionnaire. They were weighed and venous blood samples were taken for laboratory analysis of lipid, protein and haemorheological parameters. Urine nicotine and cotinine concentrations were measured[21] to confirm smoking cessation.

High density lipoprotein (HDL) was isolated by selective precipitation of apo-B lipoproteins with dextran sulphate and magnesium chloride. Total cholesterol (-C) and HDL-C were analysed enzymatically using Boehringer reagents on a Centrificem centrifugal analyser. Haematocrit (Hct) was determined by microcentrifugation at 13 000 g for 5 min. Fibrinogen concentration was measured by a thrombin clotting technique[22]. Viscosity measurements were all made on a Contraves LS 30 viscometer with a bob and cup system thermostated at 37°C. Blood viscosity measurements were made at low [0.277 s^{-1}] (LS) and high [128.5 s^{-1}] (HS) shear rates at native Hct. Because plasma shows Newtonian viscometric characteristics, its viscosity

Table 1. Biochemical and rheological results: median (range) while smoking and 2 weeks after stopping (n=30)

	Smoking	*Non-smoking*
Cholesterol (mmol/l)	5.31 (3.79-7.38)	5.35 (2.83-7.92)
HDL-cholesterol (mmol/l)	1.47 (0.78-2.03)	1.56 (0.79-2.93)**
Plasma protein (g/l)	72.9 (65.4-87.9)	70.5 (53.2-100.6)
Fibrinogen (g/l)	3.54 (2.02-5.62)	2.95 (1.61-5.84)**
Hct (%)	47.0 (41.0-58.0)	45.0 (38.0-55.0)***
ηP (mPa.s)	1.40 (1.28-1.97)	1.33 (1.25-1.68)**
ηLS (mPa.s)	46.1 (28.4-69.1)	38.1 (25.1-76.5)***
ηHS (mPa.s)	4.7 (4.0-7.1)	4.4 (3.7-6.1)**
Factor VII (% activity) (n=10)	86.0 (68-125)	92.0 (59-137)
Factor VIII (% activity) (n=10)	76.0 (37-130)	65.0 (35-142)

**$P<0.01$; ***$P<0.001$.
Hct, haematocrit; η P, plasma viscosity; ηLS, low shear whole blood viscosity; ηHS, high shear whole blood viscosity; mPa.s, milliPascal seconds.

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(P) was measured only at high shear rate. Total plasma protein concentration was measured using the Biuret method[23]. Clotting factor (Factor VII, Factor VIII) concentrations were measured by one stage assays of coagulant activity. Nicotine and cotinine concentrations were measured by capillary gas chromatography[24]. The statistical method used was the Wilcoxon signed paired rank sum test.

Results

Thirty-two chronic smokers originally agreed to participate in the study. One subject did not succeed in quitting smoking and one further subject did not return for follow-up testing. In the remaining 30 subjects, following two weeks of complete tobacco withdrawal, confirmed by the absence of urinary nicotine and cotinine, there were significant reductions in the fibrinogen, Hct, plasma viscosity and whole blood viscosity (at low and high shear rates) as well as a significant increase in HDL-C (Table 1). Plasma protein concentration did not change significantly.

In a sub-group where factors VII and VIII concentrations were measured, there were no significant changes in the two-week period after tobacco withdrawal. Weight did not significantly change throughout the study period. Over the four weeks, there was no alteration in exercise habits in any of the subjects, while most increased non-alcoholic beverages, five increased their alcohol consumption and three subjects documented a decrease. There was no qualitative increase in the fat or fibre content of the diet.

Discussion

While the exact mechanism of tobacco-induced vascular damage is at present unclear, the adverse effects of tobacco smoking on atherosclerotic disease may be mediated by both atherogenic and thrombogenic mechanisms[9-12] as well as direct damage to endothelial cells[25]. Blood viscosity[13], coagulation[14-16], fibrinogen[14], platelet aggregation[17], serum cholesterol[16] and Hct[18] are often increased in smokers compared to non-smokers, while HDL-C[19] and platelet survival[15] are commonly decreased. All these changes are often seen in patients with clinical atherosclerotic disease. From this study HDL-C, fibrinogen, haematocrit and blood viscosity improved within two weeks of complete tobacco cessation. A low HDL-C confering an increased risk of coronary artery disease is well described. In this study, HDL-C increased in the 2 week period of stopping smoking, while total cholesterol remained constant. This may be due to a loss of tobacco-associated impairment in intravascular lipolysis[19] and may also indicate an improvement in the clearance of atherogenic remnants which are catabolized in the same pathway as the formation of HDL[20].

Fibrinogen elevation, an independent cardiovascular risk factor[26-29], may be the major determinant by which the harmful effects of smoking are mediated. The pathophysiological significance of fibrinogen can be seen from its importance in facilitation of platelet aggregation and adhesion as well as its function in thrombus formation and blood viscosity. The rapid decline in the measured fibrinogen after stopping smoking may indicate that the powerful stimulus to the lung macrophages to produce hepatocyte-stimulating factor had been removed[30]. Smokers polycythaemia is a well described phenomenon[18], and elevations in haematocrit have also been associated with cardiovascular risk[29]. The reduction in haematocrit plus the further lowering of fibrinogen on quitting smoking produced a significant reduction in whole blood viscosity at both high and low shear rates. Low shear rate is of interest as it is in this region where rouleaux formation induced mainly by fibrinogen is an important influence and may also promote atherogenesis[11]. By contrast, the high shear rate viscosity, (which is influenced by plasma viscosity, Hct and also erythrocyte deformability) may produce direct endothelial damage[25]. A pronounced increase in viscosity may itself produce ischaemia. Blood flow in vessels narrowed by atheroma would also be further compromised. Whilst no significant changes were observed in clotting factors VII and VIII in this study, it is probable that a greater number of subjects would be needed to clarify whether quitting smoking has any early effect on these factors important in thrombogenesis.

Quitting smoking may provoke a variety of behavioural and dietary changes, such as an increase or decrease in exercise, calorie consumption or fluid intake. Some of these changes may potentially offset the positive benefits of tobacco withdrawal on atherogenic and thrombogenic factors. Obesity has been associated with a raised fibrinogen[31], and deterioration of blood viscosity[32], as well as a reduced HDL-C[33], while alcohol consumption and exercise appear to have the opposite effect[32-34]. The short timescale of this study possibly minimized the influence of obesity-induced effects associated with changes in exercise or behaviour on these factors, important in atherosclerotic disease.

In this study, 94% of the subjects abstained from smoking completely and this made it possible to carry out this within-subject investigation of the time course of alteration of several thrombogenic and atherogenic factors. The 4-week period was chosen to avoid influences of the menstrual cycle in the female subjects on the measured variables. Previous studies have shown that improvements in individual factors such as Hct[35] or HDL-C[36] may occur shortly after tobacco withdrawal.

This study has shown that a number of atherogenic and thrombogenic factors may be influenced in 2 weeks in the same individual by quitting smoking. These combined changes may be responsible for the reductions in the incidence of cardiovascular disease following the cessation of smoking. However, further studies are needed to assess if these alterations in both atherogenic and thrombogenic factors with cessation of smoking are maintained in the long-term[14].

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