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Smoking cessation does not change urinary albumin excretion in normal subjects

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The aim of the study was to examine the effect of smoking cessation on urinary albumin excretion (UAE) in normal subjects. The study consisted of two parts. The first was a randomized 4-week study, in which 182 heavy smokers were asked to quit smoking immediately (n=69, available for analysis) or to continue smoking for another 4 weeks (n=70, available for analysis). After 4 weeks, the latter group was also asked to stop smoking. The second part was a non-randomized follow-up study comparing UAE in 33 unsuccessful and 57 successful quitters followed for 26 weeks. Measurements of UAE (ELISA) were taken from 24-h urine samples before smoking cessation, after 4 weeks, and after 26 weeks. After 4 weeks, no statistically significant change in UAE was found within each group or between quitters and smokers. The 95% confidence intervals of the change in log UAE were −7.4 to 9.9% of the initial value in the smoker group and −4.9 to 11.3% in the quitter group. In the second part of the study, after 26 weeks, a 16% increase (95% confidence interval 5.5 to 26.5%) in mean log UAE was found in the group that had stopped smoking ($p<0.003$), but no statistically significant difference in UAE between continued smokers and quitters was found after adjusting for the baseline level (ANCOVA). In conclusion, smoking cessation seems to have no effect on UAE within the physiological range in normal subjects over an observation period of 4 weeks, and no sign of a decrease in UAE was seen after 26 weeks of smoking cessation.

Key words: albuminuria; cigarette smoking; microalbuminuria; smoking cessation; urinary albumin excretion

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It is well established that microalbuminuria (elevated albumin excretion, defined as 30–300 mg 24 h^{-1}) is associated with the progression of renal disease in Type 1 diabetes [1, 2] and is a predictor of mortality in non-insulin-dependent diabetes mellitus [3, 4]. The presence of microalbuminuria in non-diabetic patients has also attracted increased attention in recent years since it may be an indicator of cardiovascular risk, as suggested by Yudkin *et al.* [5]. A possible link between smoking and microalbuminuria has been demonstrated by a

number of studies, which have shown higher urinary albumin excretion (UAE) in both diabetic and non-diabetic smokers than in non-smokers [6–9]. The underlying mechanism is unknown, but vascular damage has been associated with both smoking and UAE.

Because of the lack of intervention studies, little is known about the effect of smoking per se on UAE in normal subjects. The aim of this study was therefore to determine the effect of smoking cessation on 24-h UAE in normal subjects. The first part of the investigation was a randomized controlled study over a 4-week period. Because it was considered unethical to let the control group continue smoking for more than 4 weeks, the second part of the study compared UAE in unsuccessful and successful quitters available at follow up after 26 weeks.

SUBJECTS AND METHODS

Initially, 200 subjects, men and women, aged 35–66, were recruited through an advertisement in a newspaper. All were heavy smokers who had smoked at least 15 cigarettes per day during the previous year. All subjects underwent an interview about their past and present medical history. The eligible subjects did not have any chronic or acute diseases and were not taking any medication. All subjects included had routine blood tests performed, including one for fasting blood sugar. The 182 included subjects were randomized by a computer-generated list either to immediate smoking cessation (quitter group, 100 subjects) or to continued smoking as usual for 4 weeks more (control group, 82 subjects) before smoking cessation. Because of deviations from the protocol, 30 subjects in the quitter group (15 of whom were still smoking after 4 weeks) and 23 subjects in the control group were excluded during the first 4-week study period, leaving a final study group at 4 weeks of 70 quitters and 69 continued smokers (Fig. 1A).

After 26 weeks, the subjects were followed-up in a non-randomized study by inviting all those from the initial study group who had completed the first part of the study (n=139). Because the observation times of the subjects in this part of the study differed only by 4 weeks

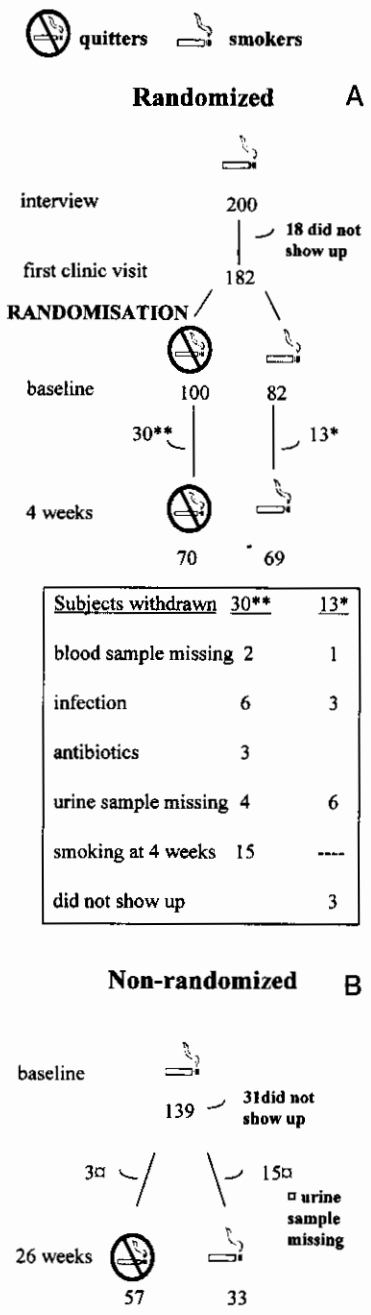

FIG. 1. Flow chart for subjects in smoking cessation study. The study was divided into two parts. A. In the first part of the study, the subjects were randomized to either smoking cessation or continued smoking for another 4 weeks. After 4 weeks, the control group was also asked to stop smoking. B. In the second part of the study, the UAEs of successful quitters and continued smokers were compared after 26 weeks, independently of the first randomization.

(with a maximum of 26 weeks), it was thought justified to consider the group as a whole, irrespective of this 18% difference in time. A total of 108 subjects returned after 26 weeks; 66 subjects were successful quitters and 42 were still smoking. No urine samples were available for 18 subjects, leaving 57 successful quitters and 33 continued smokers available for analysis. The subjects who dropped out from the study (i.e. due to lack of urine samples), were not considered different from those who completed the study (Fig. 1B).

Urine samples were collected from the subjects at baseline, after 4 weeks, and after 26 weeks. No restrictions were placed on the physical activities of daily life. The urine samples were stored frozen (at −20 °C) in polystyrene test tubes and samples were transferred to polyethylene containers before analysis. All samples were measured at the same time. Urinary creatinine was measured in all samples in an attempt to compensate for possible incompleteness in collection.

Seated blood pressure was measured using a standard sphygmomanometer after 10 min of rest. Weight and height were measured to calculate body mass index (BMI), as body weight in kg divided by height squared (m^2). Carbon monoxide in end-expiratory air and plasma cotinine concentration were measured to ensure compliance with smoking cessation.

For 12 weeks the subjects were supplied with nicotine patches releasing 15 mg of nicotine daily (Nicorette (R), LKB, Pharmacia, Helsingborg, Sweden). After that, dosage was tapered to zero during a 4-week period. The study was conducted from September 1994 to March 1995.

The study was approved by the local ethics committee and signed informed consent was obtained from each subject. Other data from this study cohort have been and will be published elsewhere [10].

Analytical methods

Urinary albumin concentrations were determined by enzyme-linked immunoadsorbant assay in 24-h urine collections. The intra-assay coefficient of variation (CV) of this assay has previously been determined as 2.1%. The corresponding CV measured between days was 8.3% [11].

Statistics

UAE rates were transformed to logarithmic values for normal distribution, for statistical testing by paired t tests within each group. Means of UAE rates are expressed as geometric means. The difference in log UAE between quitters and continued smokers was tested using ANCOVA, adjusting for baseline levels. The influence of changes in body weight and mean blood pressure (diastolic+one-third of the difference between systolic and diastolic) on log UAE was tested using ANCOVA with smoking status as an independent variable. A probability value (p) below 0.05 was considered statistically significant.

RESULTS

Table I shows the characteristics of the study population. The two intervention groups were compared at baseline. No significant difference in UAE was found within or between the two groups before and 4 weeks after smoking cessation ($p=0.7$) (Fig. 2). The 95% confidence intervals of the changes in log UAE after 4 weeks were −7.5 to 9.9% of the initial value in the smoker group and −4.9 to 11.3% in the quitter group. In the quitter group a statistically significant body weight gain of 1.8% was observed after 4 weeks ($p<0.0001$), whereas there was no weight change in the smoker group ($p=0.2$). The mean blood pressure did not change statistically significantly in the quitter group after 4 weeks ($p=0.7$), whereas in the smoker group the mean blood pressure was statistically significantly lower ($p<0.002$). Neither the change in body weight nor in mean blood pressure were significant covariates of the change in log UAE ($p=0.6$).

After 26 weeks, a 16% statistically significant increase in log mean UAE was found in the quitter group (95% confidence interval 5.5 to 26.5%) ($p<0.003$). This increase was not significant if the albumin-to-creatinine ratio was used instead ($p=0.06$). No statistically significant change in UAE in the group of continued smokers was found ($p=0.4$); the 95% confidence interval of the change was −6.8 to 17.8%; there was no statistically significant difference between the continued

TABLE I. Characteristics for all subjects in the 4-week randomized study.

	Baseline		4 weeks	
	Quitters	Continued smokers	Quitters	Continued smokers
No. of subjects	70	69		
Gender, male/female	40/30	36/33		
Age, mean±SD, years	45.9±8.5	44.2±7.6		
Weight, mean±SD, kg	76.5±13.8	73.9±12.4	77.9±13.9***	74.1±12.4
Body mass index (BMI), mean±SD, kg m^{-2}	25.2±3.5	24.1±2.9	25.6±3.6***	24.2±3.0
Blood pressure, mean±SD, mm Hg				
Systolic	131.3±17.6	126.9±15.0	128.8±15.6	122.9±15.1**
Diastolic	81.3±9.7	80.9±9.1	82.3±10.2	78.9±9.1*
Mean†	98.2±11.0	96.3±9.8	97.8±11.0	93.6±10.3*
Serum creatinine, μmol l^{-1}	83.4±11.3	83.3±12.9		
No. of cigarettes per day	22.6±6.5	23.8±6.6	0	22.7±7.8
Plasma cotinine, ng ml^{-1}	278.1±123.1	277.0±93.8	114.2±71.3***	283.1±93.6
Urinary albumin excretion (UAE), geometric mean (range), mg $24h^{-1}$	5.1 (0.8–81.6)	5.6 (1.5–138.6)	5.3 (1.2–68.0)	5.8 (1.2–115.2)
% change of log UAE over 4 weeks, 95% confidence intervals	−4.9 to 11.3	−7.4 to 9.9		

† Diastolic+one-third of the difference between systolic and diastolic.
*p<0.03, **p<0.004 and ***p<0.0001, significantly different from baseline value.

smoker and quitter groups after baseline adjustment using ANCOVA (p=0.3) (Table II and Fig. 3).

After 26 weeks a statistically significant increase in body weight was observed in the quitter group (6.4%; p<0.00001) and in the continued smoker group (3%; p<0.0006). The mean blood pressure did not change statistically significantly in any of the groups (p=0.18 in the quitter group and p=0.7 in the continued smoker group). The change in body weight after 26 weeks was not a significant covariate of the change in log UAE (p=0.9).

DISCUSSION

In this randomized design study, urinary albumin excretion (UAE) did not change after 4 weeks of smoking cessation in healthy subjects. At follow-up after 26 weeks, the UAE of smokers and successful quitters was compared in a non-randomized design, and no statistically significant difference between the two groups was found. To our knowledge, this is the first randomized longitudinal study (4 weeks) investigating the influence of smoking cessation on UAE in normal subjects.

Because of the relatively short observation period, the present study cannot rule out the possibility that smoking increases UAE as a long-standing or even irreversible phenomenon. The findings of our study are in accordance with the previous failure to demonstrate any acute effect on albuminuria in diabetic patients subjected to heavy smoking over a 5.5-h period [12]. Similarly, no difference in smoking habits could be demonstrated between progressors and non-progressors in a 5-year follow-up study of diabetic patients with microalbuminuria [13]. On the other hand, in that study microalbuminuria was found to be significantly higher in the smoking group than in the non-smoking group.

Reversibility of UAE has been found after smoking cessation in diabetic patients [2, 6, 14]. Those studies, however, are observational, non-randomized studies, comparing smokers and non-smokers. Moreover, their designs did not allow estimation of a period required for any decrease in UAE to occur. In a cross-sectional, non-randomized study of non-diabetic subjects, Gosling & Beavers found higher UAE in

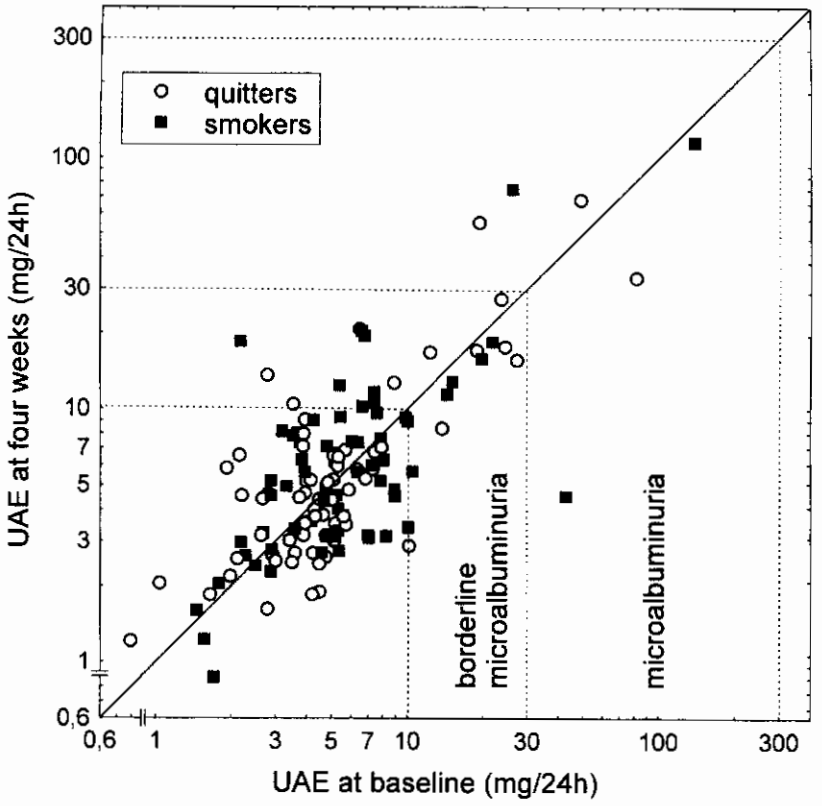

FIG. 2. Plot of 24-h UAE at baseline against 24-h UAE at 4 weeks in all subjects (n=139).

smokers than in non-smokers, whereas no correlation was found between the number of cigarettes smoked per day and UAE [15].

The possible mechanisms of tobacco-induced, elevated UAE rate may be increased vascular permeability, increased heart rate and blood pressure when smoking, decreased oxygen delivery to the glomeruli and increased glomerular filtration rate [8]. Moreover, smoking may also induce elevated UAE through the catecholamine response which raises systemic and, perhaps, intraglomerular blood pressure [16]. Microalbuminuria has been shown to be dependent on blood pressure [17]. In our study, blood pressure did not change significantly following smoking cessation or when nicotine patches were discontinued in the smoking cessation group. However, blood pressure may still be an important factor in the development of microalbuminuria. Other studies indicate that the long-term effect of smoking induces vascular dysfunction by increasing the transcapillary escape of albumin [18]. These effects may not be reversible shortly after smoking cessation. In the smokers' group the slight decrease observed may be due to the effect of habituation to blood pressure recording by the investigators [19].

The elevated plasma cotinine concentrations in the quitters' group are most likely to be due to the use of nicotine patches and therefore it cannot be ruled out that the use of nicotine patches may have obscured differences between the two groups.

The observed increase in albuminuria in quitters may be due to seasonal, dietary or unknown factors. Thus, there was no difference between unsuccessful and successful quitters at 26 weeks. It has previously been suggested that lifestyle factors, such as alcohol consumption, weight gain, exercise and infections can influence UAE [20]. The body weight gain in relation to smoking cessation has been studied extensively before and is linked to an increase in caloric intake and a decrease in resting metabolic rate [21, 22]. In

TABLE II. Urinary albumin excretion (UAE), blood pressure and weight at baseline and 26 weeks after smoking cessation in non-randomized study.

	Baseline		26 weeks	
	Quitters	Continued smokers	Quitters	Continued smokers
No. of subjects	57	33		
Weight, mean±SD, kg	76.0±13.9	73.0±10.15	80.9±14.7†	74.7±10.1††
Blood pressure, mean±SD, mmHg				
Systolic	130.2±19.1	125.4±12.2	130.4±20.1	123.4±13.2
Diastolic	81.3±10.6	79.4±8.4	84.3±9.8*	79.0±9.2
Mean§	97.7±12.9	94.5±8.4	99.7±12.6	93.8±10.0
UAE, geometric mean (range), mg 24 h^{-1}	5.0 (1.5–138.6)	5.6 (0.8–81.6)	6.6 (1.2–278.2)**‡	6.1 (1.0–48.5)NS

§Diastolic+one-third of the difference between systolic and diastolic.
*p=0.03; **p=0.003; †p<0.00001; ††p<0.0006; significantly different from baseline within the group.
‡No significant difference between smokers and quitters as regards UAE at 26 weeks (ANCOVA, p=0.27).
NS, no significant difference from baseline value within the group (p=0.4).

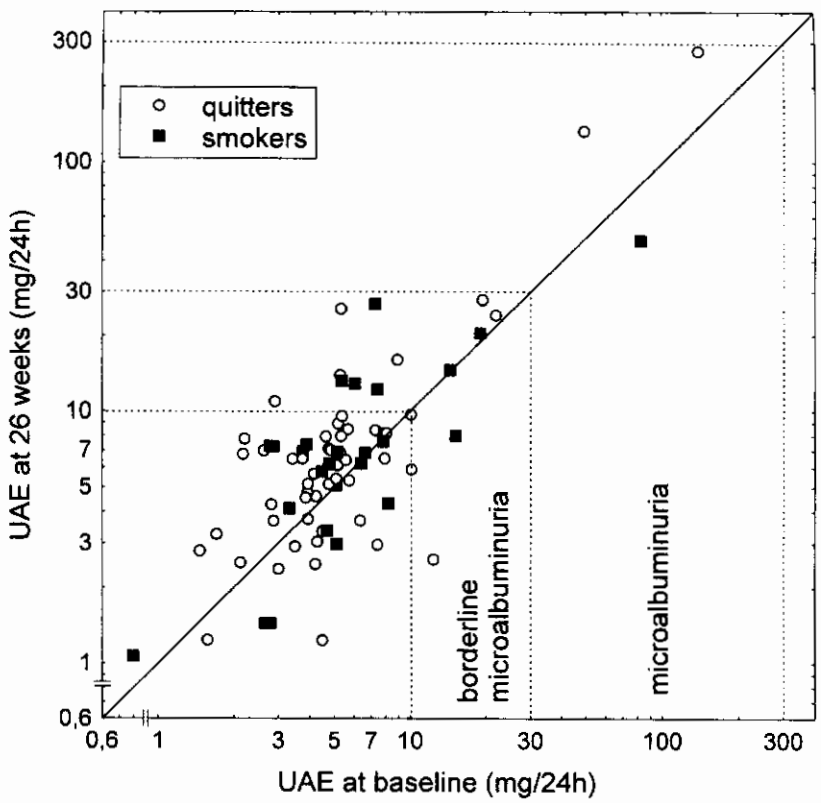

FIG. 3. Plot of 24-h UAE at baseline against 24-h UAE at 26 weeks in all subjects (n=90).

our study we found a moderate increase in weight in the quitter group. This weight gain may have obscured a beneficial effect from smoking cessation on UAE. However, there was no correlation between that increase and the increase in UAE.

Our urinary samples were analysed in one batch, which meant that there were different times of storage at −20 °C. Albumin has previously been reported to be very stable in urine samples [23]. However, in another study that evaluated the effects of freezing urine, falsely low results were found after prolonged storage [24]. Thus, it is possible that less albumin had been trapped in the 26-week samples than in the baseline and 4-week samples. Our sampling technique was the same throughout the study, suggesting that any errors introduced by the transfer of urine from one type of test tube to another were of the same order of magnitude.

In conclusion, although the use of nicotine patches may have influenced the results, these data indicate that smoking cessation has no effect on UAE within the physiological range in normal subjects over an observation period of 4 weeks. After 26 weeks there was a small but statistically significant increase in albuminuria in the subjects who had stopped smoking for at least 26 weeks the cause of which is unclear and which is probably not clinically important. The data appear to rule out the possibility that smoking cessation induces a decrease in UAE within 26 weeks.

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