

# Cigarette Smoking and Progression of Retinopathy and Nephropathy in Type 1 Diabetes

I. Mühlhauser, R. Bender, U. Bott, V. Jörgens, M. Grüsser, W. Wagener, H. Overmann, M. Berger

Department of Nutrition and Metabolic Diseases, Heinrich-Heine University, Düsseldorf, Germany

The objective of the present study was to analyse the association between cigarette smoking and progression of retinopathy and nephropathy, respectively, in a prospective multicentre study including 636 people with Type 1 diabetes: 81 % of the original cohort of consecutively referred patients, aged 15 to 40 years and free of severe late diabetic complications. At baseline, all patients had participated in a 5-day in-patient group treatment and teaching programme for intensification of insulin therapy. Patients were examined at recruitment, and after 1, 2, 3 and 6 years including assessment of smoking status, blood pressure, metabolic control, and degree of nephropathy. Degree of retinopathy was assessed by ophthalmoscopy or fundus photography at baseline and after 6 years. Several logistic regression analyses were performed by describing the responses retinopathy and nephropathy, respectively, either as progression yes/no or as actual status at the 6-year follow-up and by using different measures for smoking. Adjustments for important covariables were made. While significant associations between smoking, and retinopathy and nephropathy respectively, were found, the relations were variable depending on the statistical model used. The results show that the real associations between smoking and retinopathy and nephropathy are complex and that more emphasis should be put on the complete description of the response variables and the statistical models used in clinical and epidemiological research.

**KEY WORDS** Smoking Nephropathy Retinopathy Intensified insulin therapy Education Glycaemic control Epidemiology Statistics Logistic model

## Introduction

During recent years evidence has been accumulating that cigarette smoking could be involved in the development and progression of nephropathy and retinopathy.<sup>1-5</sup> However, results have been inconclusive, especially with respect to retinopathy. Most studies investigating the influence of smoking on microvascular complications have been cross-sectional in design.<sup>1,2,4,5</sup> Due to selective mortality, that is smoking patients die earlier than non-smoking patients, such studies are likely to miss or underestimate a possible association between smoking and nephropathy or retinopathy. In addition, in many studies on the course of microvascular complications smoking status of the patients has not been mentioned at all,<sup>1,6,7</sup> or only limited information was available on smoking history.<sup>1,8</sup>

The impact of smoking on microvascular complications in diabetes cannot be assessed by randomized controlled trials. Therefore, the best available approach is to use epidemiological models to describe the association

between smoking and retinopathy or nephropathy as accurately as possible. The lack of a statistically significant association between smoking and retinopathy or nephropathy could be due either to the lack of a real association, low statistical power, or to the inadequacy of the statistical model used to describe a real association. In addition, the association between smoking and the development and progression of microvascular complications could be complex and is confounded by other covariables and therefore, it could be impossible to characterize it by a single statistical model. Notably, previous publications did not report the adequacy of their statistical models.

In the present study, we have prospectively analysed the relations between cigarette smoking and progression of nephropathy and retinopathy over a period of 6 years in a large cohort of persons with Type 1 (insulin-dependent) diabetes.

## Patients and Methods

The study population consisted of 636 persons with Type 1 diabetes (81 % of the original cohort) who had taken part in a German multicentre study, which documented the feasibility to translate an intensified insulin treatment

Correspondence to: Dr Ingrid Mühlhauser, Medizinische Klinik der Universität Düsseldorf, Klinik für Stoffwechselkrankheiten und Ernährung, Moorenstraße 5, D-40225 Düsseldorf, Germany

and teaching programme (TTP) from a specialized University diabetes centre to general internal medicine departments.<sup>9-11</sup> Since the outcome was comparable for the specialized centre and the nine participating general hospitals, for the purpose of the present study the combined group of 636 patients was analysed. Detailed descriptions of the study population, the TTP, translation of the TTP, medical care of the patients after discharge, evaluation protocols, drop outs, and results for up to 6 years of follow-up have been published.<sup>9-11</sup> In short, 784 consecutively referred persons with Type 1 diabetes, aged 15-40 years, and free of severe late diabetic complications (serum creatinine  $>177 \mu\text{mol l}^{-1}$ , blindness) had taken part in the same 5-day in-patient TTP for intensified insulin therapy in one of the 10 participating hospitals and were re-examined after 1, 2, 3, and 6 years.

Baseline data were collected by the participating physicians at the local hospitals, whereas the follow-up examinations were performed by four investigators (V.J., M.G., W.W., H.O.), who had not been involved in the care of the patients. At the 1, 2, and 3-year follow-up, patients were either examined at their respective hospitals or visited at their homes (e.g. 43% home visits at the 3-year follow-up examination). The 6-year follow-up was performed using a mobile ambulance as described elsewhere.<sup>12</sup> The van was converted to allow the mounting of a non-mydratic CR4-45NM eye camera (Canon Europe, Amstelveen, The Netherlands). At baseline, the usual clinical and diabetes-related examinations and laboratory measurements were performed in each hospital using standard methods. At each examination venous blood samples and a freshly voided urine sample were taken from each patient and transported on ice to the laboratory of the reference centre (Düsseldorf Hospital). At baseline and at the 1, 2, and 3-year follow-up, HbA<sub>1c</sub> was measured in all patients using a microcolumn method (Boehringer Mannheim, Mannheim, Germany), with a normal non-diabetic range of 5.6-7.4%. At 3 years, HbA<sub>1c</sub> and HbA<sub>1c</sub> levels were measured simultaneously, and at 6 years, only HbA<sub>1c</sub> was measured by the Diamat<sup>®</sup> HPLC-method (Biorad, München, Germany) (reference range 4.3%-6.1%). For the purpose of the present study HbA<sub>1c</sub> values were converted into HbA<sub>1c</sub> values and the latter were used for analysis ( $\text{HbA}_{1c} = -0.279 + 0.864 \text{HbA}_{1c}$ ,  $R^2 = 0.9$ ). Protein concentration in the urine was measured by the laser turbidimetric method.<sup>13</sup> Sitting blood pressure was measured using a mercury sphygmomanometer as described previously.<sup>12</sup>

At baseline, in the nine general hospitals retinal status was assessed by the consultant ophthalmologist of the respective hospitals, and in the Düsseldorf hospital either by a consultant ophthalmologist or by the non-mydratic CR4-45NM eye camera as previously described.<sup>12</sup> At the 6-year follow-up, non-mydratic photography was performed in the mobile ambulance. In all subjects a macula centred photograph was obtained of one eye. If

there was any hint of retinopathy on this first photograph, a picture of the other retina was taken as well. All photographs were examined by two independent experts in Düsseldorf. In addition, patients were handed out a "Diabetic Retinopathy Examination Chart"<sup>14</sup> modified according to the recommendations of the "European Retinopathy Working Party",<sup>15</sup> and patients were asked to consult their ophthalmologists and to send back one copy of the filled in examination chart to the Düsseldorf Study Centre. All available eye examination results were used in order to rate the degree of retinopathy for each patient. In case the degree of retinopathy differed between both eyes, and in the case the findings between methods differed, the higher degree of retinopathy was used. At the 6-year follow-up, best visual acuity was assessed in the van using the visual acuity charts of Ferris.<sup>16</sup>

Patients were grouped according to the degree of retinopathy:<sup>15</sup> level 1: no retinopathy; level 2: non-proliferative retinopathy without macular involvement, no history of laser therapy for retinopathy; level 3: pre-proliferative and proliferative retinopathy, macula involvement, macula oedema, history of laser therapy, advanced diabetic eye disease;<sup>15</sup> level 4: blindness of one or both eyes due to diabetes (defined as legal blindness or best visual acuity  $\leq 0.1$ ). Progression of retinopathy was assumed if a patient had progressed to any higher level of retinopathy at follow-up.

In addition, patients were grouped according to renal parameters: level 1: proteinuria  $\leq 50 \text{ mg l}^{-1}$  and serum creatinine  $\leq 133 \mu\text{mol l}^{-1}$ ; level 2: proteinuria 51-499  $\text{mg l}^{-1}$ , and serum creatinine  $\leq 133 \mu\text{mol l}^{-1}$ ; level 3: proteinuria  $\geq 500 \text{ mg l}^{-1}$  and serum creatinine  $\leq 133 \mu\text{mol l}^{-1}$ ; level 4: serum creatinine  $>133 \mu\text{mol l}^{-1}$ ; level 5: renal replacement therapy. Progression of nephropathy was assumed if a patient had progressed to any higher level according to these renal parameters.

Smoking habits were assessed at each examination. Patients were asked whether they smoke at present and, on average, about how many cigarettes per day they smoke at present, whether they have smoked a year ago, and on average about how many cigarettes per day they have smoked a year ago, and when they had started to smoke. Life-time cigarette pack years until the last follow-up examination, cigarette pack years for diabetes duration until the last follow-up examination and cigarette pack years for the period of the 6 years of follow-up were estimated for each patient. Ex-smokers were defined as smokers who reported to be non-smokers on at least 2 yearly follow-up examinations or, in case of the 6 year follow-up, to have stopped smoking for at least 1 year. Heavy cigarette smoking was defined as reported cigarette smoking of at least 20 cigarettes per day.

### Statistical Analysis

For comparisons of groups the Wilcoxon rank sum test (continuous response) and the exact test of Fisher (categorical response) were used. In order to investigate

the associations between smoking and retinopathy and nephropathy respectively, with an adjustment for important confounding factors, various multiple regression models were performed. As response variables 'change of retinopathy', 'change of nephropathy' as well as retinopathy and nephropathy status at follow-up were used. Smoking was measured by means of four different variables: 'life-time cigarette pack years', 'diabetes duration cigarette pack years', 'study period cigarette pack years', and 'heavy smoking (yes/no)'. As confounding factors the variables diabetes duration, initial HbA<sub>1c</sub> levels, mean HbA<sub>1c</sub> levels during follow-up (mean of the values of the 1, 2, 3, and 6-year follow-up examination), mean systolic blood pressure, mean diastolic blood pressure (mean of all values: baseline, 1, 2, 3, and 6-year follow-up examination), gender (male/female), and age were used in all models. For the response retinopathy/nephropathy status at follow-up an additional adjustment for the corresponding status as baseline was made. We used two different HbA<sub>1c</sub> variables because there was a significant improvement of HbA<sub>1c</sub> levels after intensification of insulin therapy.<sup>9-11</sup>

It should be noted that the risk of disease progression of both retinopathy and nephropathy increases until 25 years of diabetes duration and decreases with longer diabetes duration. That means that a standard regression model containing only the linear term of diabetes duration cannot describe the relationship between disease progression and diabetes duration adequately. The easiest way to yield a better description of such an association is to include at least the quadratic term of diabetes duration in the regression equation. We tested the quadratic effect of diabetes duration in various models and found that it was sometimes highly significant with a clear improvement of model adequacy but sometimes not. As the consideration of quadratic effects makes it more difficult to interpret the regression coefficients we decided to drop the quadratic term of diabetes duration from this analysis. Moreover, the goal of the present paper was not the investigation of the relationship between diabetes duration and disease but the relationship between smoking and disease by considering diabetes duration only as a confounding factor.

Since all response variables were first of all measured

in an ordinal scale the proportional odds model<sup>17</sup> was applied. However, we found by means of a score test that the proportional odds assumption was not fulfilled in all cases, indicating that the association between disease and smoking cannot be characterized by a single odds ratio. Hence, we used the following binary variables as response: progression of retinopathy (yes/no), any retinopathy at follow-up (yes/no), advanced retinopathy at follow-up (yes/no), progression of nephropathy (yes/no), any nephropathy at follow-up (yes/no), at least macroproteinuria at follow-up (yes/no). Therefore, considering six response variables and four different smoking variables in the final stage of the analysis  $6 \times 4 = 24$  different binary logistic regression models were performed.

Due to the observational character of the study no adjustment for multiple hypotheses testing was made. For computations the SAS procedures MEANS, TABULATE,<sup>18</sup> NPARTWAY,<sup>19</sup> and LOGISTIC<sup>20</sup> were used.

## Results

Vital status was available for all 784 patients except for 9, who could not be traced. Thirteen patients had died. A total of 135 patients either declined to participate ( $n = 57$ ) or had moved away too far to be re-examined. Compared to the 636 re-examined patients the 135 drop outs had higher HbA<sub>1c</sub> levels before the TTP ( $8.8 \pm 1.9\%$  vs  $8.3 \pm 1.8\%$ , mean  $\pm$  SD,  $p = 0.003$ , Student's *t*-test) and a lower incidence of severe hypoglycaemia during the year before the TTP (0.15 vs 0.28 cases per patient,  $p = 0.031$ , Wilcoxon rank sum test); there were no differences with respect to smoking habits, nephropathy or retinopathy.

At the 6-year follow-up examination, 279 (43.9% of the total of 636 patients) were non-smokers, 296 (46.5%) were smokers (22 patients had started smoking during the 6-year study period), and 61 patients (9.6%) were ex-smokers (14 patients had stopped smoking before recruitment and 47 had stopped smoking during the study period). Among smokers, 151 (51%) patients were heavy smokers. Among smokers and ex-smokers, 52% had  $\leq 10$ , 30% had between 11 and 20, and 18% had  $> 20$  life-time cigarette pack years.

Data on retinopathy for both study time points (baseline

Table 1. Cross-tabulation for degree of retinopathy

Retinopathy at baseline	Retinopathy after 6 years			
	None ( $n = 388$ )	Non-proliferative ( $n = 118$ )	Advanced ( $n = 107$ )	Blind* ( $n = 10$ )
None ( $n = 500$ )	369	98	33	0
Non-proliferative ( $n = 74$ )	19	20	30	5
Advanced ( $n = 39$ )	0	0	34	5

Figures indicate number of patients

\*One or both eyes.

and after 6 years) were available for 613 patients. The distribution of patients according to the degree of retinopathy is summarized in Table 1. Of the 13 deceased patients 5 were known to have had advanced retinopathy. Visual acuity was assessed in 627 patients. In 9 patients best visual acuity was moderately reduced ( $<0.5 > 0.25$ ), in 6 severely reduced ( $\leq 0.25 > 0.1$ ), 6 patients were blind in one eye and 4 patients were blind in both eyes (best visual acuity  $\leq 0.1$ ). At baseline, 40 of the total group of 636 patients (6.3%), and at 6 years, a cumulative of 74 patients (11.6%) had a history of laser therapy for retinopathy. Change of the level of retinopathy from baseline to the 6-year follow-up: no change 423 (69%) patients, worsening by 1 level 133 (21.7%) and by 2 levels 38 (6.2%) patients, improvement by 1 level 19 (3.1%). In Table 2 patients without progression are compared to those with progression of retinopathy.

Various multiple logistic regression models for retinopathy were performed:

1. Logistic regression analysis for 'progression of retinopathy' calculated as progression yes/no. Using this model 'progression of retinopathy' was significantly associated with diabetes duration, baseline HbA<sub>1c</sub>, mean HbA<sub>1c</sub> during follow-up, mean diastolic blood pressure, and all four measures of smoking status ('life time cigarette pack years', 'diabetes duration cigarette pack years', 'study period cigarette pack years', 'heavy smoking'), whereas sex, age, and systolic blood pressure were not significantly associated with progression of retinopathy (Table 3). However, using the variable 'life-time cigarette pack years' the model adequacy was poor as reflected by a low Hosmer-Lemeshow goodness-of-fit ( $p = 0.057$ ), which means that by applying this model the

association of predicted probabilities and observed responses was low. The model was more adequate using the variables 'diabetes duration cigarette pack years' (goodness-of-fit  $p = 0.26$ , Table 3), 'study period cigarette pack years' (goodness-of-fit  $p = 0.35$ ), and 'heavy smoking' (goodness-of-fit  $p = 0.39$ ), respectively. The adequacy of these four models could be increased by including the quadratic effect of diabetes duration.

- 2.(a) Logistic regression for retinopathy at the 6-year follow-up using binary responses (retinopathy yes/no) with adjustment for retinopathy status at baseline (reference category: no retinopathy). These models had an adequate goodness-of-fit (all  $p$ -values  $> 0.3$ ), and there was a significant association between 'diabetes duration cigarette pack years' ( $p = 0.039$ , difference for OR: 10 pack years, OR = 1.36, 95% CI 1.02-1.81), whereas there were non-significant associations between 'life time cigarette pack years' ( $p = 0.13$ , OR = 1.18, CI 0.95-1.48), 'study period cigarette pack years' ( $p = 0.052$ , OR = 2.06, CI 0.99-4.3) and 'heavy smoking' ( $p = 0.12$ , OR = 1.46, 0.91-2.35).
- (b) For the subgroup of patients without retinopathy at baseline (retinopathy level 1,  $n = 490$ ) logistic regression for any retinopathy at the 6-year follow-up using binary responses (retinopathy yes/no) goodness-of-fit was adequate (all  $p$ -values  $> 0.5$ ), and in these models, associations between smoking and retinopathy were of borderline significance 'life-time cigarette pack years' ( $p = 0.2$ , OR = 1.17, CI 0.92-1.48), 'diabetes duration cigarette pack years' ( $p = 0.077$ , OR = 1.33, CI 0.97-1.82), 'study period cigarette pack years' ( $p = 0.071$ ,

Table 2. Comparison of patients with and without progression of retinopathy

Variable	Progression (n = 171)	No progression (n = 442)	p
Age (yr) <sup>a</sup>	33.6 ± 7	32.7 ± 6.9	n.s.
Women (%)	49	48	n.s.
Diabetes duration (yr) <sup>a</sup>	17.6 ± 6.4	14 ± 7.5	0.0001
Baseline HbA <sub>1c</sub> (%)	8.9 ± 2	8.1 ± 1.8	0.0001
Mean HbA <sub>1c</sub> (%) <sup>b</sup>	8.1 ± 1.3	7.5 ± 1.3	0.0001
Mean SBP (mmHg) <sup>c</sup>	143 ± 15	139 ± 14	0.0018
Mean DBP (mmHg) <sup>c</sup>	83 ± 8	80 ± 8	0.0001
Life time cigarette pack years <sup>a</sup>	8.7 ± 10.7	6.3 ± 9.7	0.0021
Diabetes duration cigarette pack years <sup>a</sup>	7.0 ± 8.2	4.3 ± 6.6	0.0001
Study period cigarette pack years	2.8 ± 3.0	2.1 ± 2.8	0.0079
Heavy smokers (%)	33	23	0.018

Data are means ± SD; n.s., not significant.

DBP, diastolic blood pressure; SBP, systolic blood pressure.

<sup>a</sup>At the 6 year follow-up.

<sup>b</sup>Mean of values assessed at 1, 2, 3, and 6 years of follow-up.

<sup>c</sup>Mean of values assessed at recruitment 1, 2, 3, and 6 years of follow-up.

Total number of patients does not add up to  $n = 636$  due to missing values.

Table 3. Logistic regression for progression of retinopathy

Variables	Regression coefficient (SE)	p	Standardized coefficient	Difference for odds ratio	Odds ratio (95% CI)
Diabetes duration (yr) <sup>a</sup>	0.063 (0.014)	0.0001	0.25	5 years	1.37 (1.19–1.57)
Baseline HbA <sub>1c</sub> (%)	0.157 (0.056)	0.0048	0.17	1%	1.17 (1.05–1.31)
Cigarette pack years <sup>b</sup>	0.037 (0.014)	0.0075	0.14	10 years	1.44 (1.10–1.88)
Mean HbA <sub>1c</sub> (%) <sup>c</sup>	0.199 (0.083)	0.016	0.15	1%	1.22 (1.04–1.44)
Mean DBP (mmHg) <sup>d</sup>	0.041 (0.018)	0.023	0.17	5 mmHg	1.23 (1.03–1.46)
Male	-0.225 (0.204)	0.27	-0.06	yes or no	0.8 (0.54–1.19)
Age (yr) <sup>a</sup>	-0.005 (0.016)	0.73	-0.02	10 years	0.95 (0.70–1.29)
Mean SBP (mmHg) <sup>d</sup>	-0.004 (0.01)	0.71	-0.03	5 mmHg	0.98 (0.89–1.08)

<sup>a</sup>At the 6 year follow-up.

<sup>b</sup>Diabetes duration cigarette pack years.

<sup>c</sup>Mean of values assessed at 1, 2, 3, and 6 years of follow-up.

<sup>d</sup>Mean of values assessed at recruitment 1, 2, 3, and 6 years of follow-up.

DBP, diastolic blood pressure; SBP, systolic blood pressure.

Hosmer-Lemeshow goodness-of-fit:  $p = 0.26$ .

OR = 2.06, CI 0.94–4.49), 'heavy smoking' ( $p = 0.12$ ; OR = 1.49; CI 0.90–2.47). The  $p$ -values are slightly higher in this analysis due to the lower sample size.

- (c) For the subgroup of patients free of advanced retinopathy at baseline (combined levels 1 and 2,  $n = 560$ ) logistic regression for advanced retinopathy (combined levels 3 and 4) at the 6-year follow-up using binary responses (advanced retinopathy yes/no) the goodness-of-fit of the models was adequate (all  $p$ -values  $> 0.4$ ), but in none of the models was smoking significantly associated with development of advanced retinopathy ( $p > 0.4$ ).

Data on renal parameters for both study time points were available for 601 patients. The distribution of patients according to the degree of nephropathy is summarized in Table 4. Of the 13 deceased patients 3 had renal replacement therapy and 2 had macroproteinuria.

Change of level of nephropathy: no change 430 (71.5%) patients, worsening by 1 level 94 (15.6%), by 2 levels 14 (2.3%), by 3 levels 6 (1%), and by 4 levels 1 (0.2%) patients, improvement by 1 level 54 (9%), by 2 levels 1 (0.2%), and by 3 levels 1 (0.2%) patients. At baseline, 22 patients (3.2%) and after 6 years, 62 patients (9.9%) were treated with antihypertensive drugs. In Table 5 patients without progression are compared to those with progression of nephropathy. Various multiple logistic regression models for nephropathy were performed similar to those for retinopathy. The only model in which cigarette smoking was significantly associated with nephropathy was the logistic regression analysis for progression of nephropathy using the variable 'life-time cigarette pack years' ( $p = 0.048$ ). This model had an adequate goodness-of-fit ( $p = 0.84$ ; Table 6). However, due to multiple testing, this result should not be overinterpreted.

Table 4. Cross tabulation for degree of nephropathy

Nephropathy at baseline	Nephropathy after 6 years				
	None ( $n = 44$ )	Microproteinuria ( $n = 104$ )	Macroproteinuria ( $n = 33$ )	Increased s.-creatinine ( $n = 11$ )	Renal replacement ( $n = 9$ )
None ( $n = 480$ )	391	76	9	3	1
Microproteinuria ( $n = 95$ )	51	25	13	3	3
Macroproteinuria ( $n = 19$ )	1	3	11	2	2
Increased s.-creatinine ( $n = 7$ )	1	0	0	3	3

Results are number of patients.

Table 5. Comparison of patients with and without progression of nephropathy

Variable	Progression (n = 115)	No progression (n = 486)	p
Age (yr) <sup>a</sup>	32.9 ± 7	32.9 ± 6.8	n.s.
Women (%)	44	48	n.s.
Diabetes duration (yr) <sup>a</sup>	15.6 ± 7	14.7 ± 7.5	n.s.
Baseline HbA <sub>1c</sub> (%)	9.2 ± 2.1	8.1 ± 1.8	0.0001
Mean HbA <sub>1c</sub> (%) <sup>b</sup>	8.2 ± 1.6	7.5 ± 1.2	0.0001
Mean SBP (mmHg) <sup>c</sup>	144 ± 15	139 ± 14	0.0002
Mean DBP (mmHg) <sup>c</sup>	84 ± 8	80 ± 8	0.0001
Life time cigarette pack years <sup>a</sup>	9.2 ± 11.4	6.6 ± 9.8	0.021
Diabetes duration cigarette pack years <sup>a</sup>	6.5 ± 8.0	4.7 ± 7.0	0.018
Study period cigarette pack years	2.8 ± 3.1	2.2 ± 2.9	0.057
Heavy smokers (%)	32 %	24 %	0.098

Data are means ± SD, n.s. = not significant.  
 DBP, diastolic blood pressure; SBP, systolic blood pressure.  
<sup>a</sup>At the 6-year follow-up  
<sup>b</sup>Mean of values assessed at 1, 2, 3, and 6 years of follow-up.  
<sup>c</sup>Mean of values assessed at recruitment, 1, 2, 3, and 6 years of follow-up.  
 Total number of patients does not add up to n = 636 due to missing values.

Table 6. Logistic regression analysis for progression of nephropathy

Variables	Regression coefficient (SE)	p	Standardized coefficient	Difference for odds ratio	Odds ratio (95% CI)
Baseline HbA <sub>1c</sub> (%)	0.201 (0.06)	0.0007	0.22	1%	1.22 (1.09-1.38)
Mean DBP (mmHg) <sup>a</sup>	0.063 (0.02)	0.0024	0.27	5 mmHg	1.37 (1.12-1.68)
Mean HbA <sub>1c</sub> (%) <sup>b</sup>	0.226 (0.09)	0.014	0.16	1%	1.25 (1.05-1.5)
Cigarette pack years <sup>c</sup>	0.023 (0.01)	0.049	0.13	10 yr	1.27 (1.01-1.6)
Age (yr)	-0.020 (0.02)	0.3	-0.08	10 yr	0.81 (0.57-1.19)
Mean SBP (mmHg) <sup>a</sup>	0.004 (0.01)	0.73	0.03	5 mmHg	1.02 (0.91-1.14)
Diabetes duration (yr)	0.006 (0.02)	0.72	0.02	5 yr	1.03 (0.88-1.21)
Male	-0.011 (0.23)	0.96	-0.004	yes or no	0.99 (0.62-1.57)

<sup>a</sup>Mean of values assessed at recruitment, 1, 2, 3, and 6 years of follow-up.  
<sup>b</sup>Mean of values assessed at 1, 2, 3, and 6 years of follow-up.  
<sup>c</sup>Life time cigarette pack years.  
 DBP, diastolic blood pressure; SBP, systolic blood pressure.  
 Hosmer-Lemeshow goodness-of-fit: p = 0.84.

Discussion

This is the first analysis of the relationship between smoking and progression of retinopathy and nephropathy, respectively, of a prospectively studied large cohort of persons with Type 1 diabetes with repeated assessment of smoking status, HbA<sub>1c</sub> levels, and blood pressure during a follow-up period of as much as 6 years. The results of this study show that the relationship between smoking and retinopathy and smoking and nephropathy, respectively, is complex and cannot be explained by one single statistical model.

Various multiple logistic regression analyses were performed by describing the responses retinopathy/nephropathy either as progression yes/no or as actual status at follow-up and by using various measures of smoking. While significant associations were found between smoking and retinopathy and nephropathy,

respectively, the relations were variable depending on the statistical model used. In addition, various models were poor as reflected by a low Hosmer-Lemeshow goodness-of-fit, indicating that neither simple logistic regression analysis nor simple proportional odds models could be used in order to describe the relationship between smoking and retinopathy or nephropathy. A crucial issue is the selection of confounding factors and whether quadratic effects should be included or not. It is of note that, in previous publications, assessments of goodness-of-fit were not reported. Thus, the lack of associations between smoking and retinopathy or nephropathy could be simply due to the inadequacy of the statistical models used. In the present study, we have applied several statistical models using binary responses. The disadvantage of the binary responses is that there are only two categories, e.g. 'progression of retinopathy/nephropathy' 'yes' or 'no', which means that

patients with improvement are subsumed in the category 'no progression'. Moreover, the risks for progression may be dependent on the different disease stages. To analyse the transition times between disease stages the mathematical framework of multistate stochastic processes can be used.<sup>21,22</sup> However, these models are complex, the data requirement is particularly strict, and computer programs must become more accessible.

We tried to improve the adequacy of the statistical models by a more precise definition of smoking. Instead of just classifying patients as 'smokers', 'ex-smokers' or 'non-smokers', or using 'life-time cigarette pack years' we additionally used the variables 'diabetes duration cigarette pack years', 'study period cigarette pack years' and 'heavy smoking'. This approach resulted in an improvement of the statistical models for retinopathy. Consequently, the strongest association was found between 'progression of retinopathy' and 'diabetes duration cigarette pack years', whereas for nephropathy the strongest association was found between 'progression of nephropathy' and 'life-time cigarette pack years'. Subgroup analysis further supported the concept of a complex association between smoking and retinopathy. Thus, even the statistical models used in the present study seem inadequate in order to precisely describe the association between smoking and retinopathy or nephropathy.

The present study has several limitations, which additionally could obscure real associations between smoking and retinopathy or nephropathy. Thus, the primary goal of the present study was not the investigation of factors influencing the development and progression of microvascular complications. Therefore, assessment methods for retinopathy and nephropathy were rather crude and study conditions were not identical at the various examination time points. Proteinuria could be measured only in spot urine samples rather than in overnight or 24-h urine samples. This could explain some of the discrepancies found with respect to smoking and nephropathy between this study and previous studies.<sup>1</sup> In addition, only about 81% of the original cohort could be analysed. As expected, the prevalence of severe microvascular complications was high in the deceased patients. The impact of smoking in the patients who declined to participate and the deceased patients could not be assessed. Finally, it has to be stressed that the associations between smoking and retinopathy or nephropathy may not be causal.

In conclusion, the present study indicates that the associations between cigarette smoking and retinopathy and nephropathy, respectively, in persons with Type 1 diabetes are complex and cannot be explained by a single statistical model or by one single measure such as an odds ratio. Future studies should report the adequacy of the statistical models used.

## Acknowledgements

The study was supported by Boehringer-Mannheim, Germany, and the P. Klöckner Stiftung, Duisburg, Germany (grants to M. Berger).

## References

- Mühlhauser I. Cigarette smoking and diabetes: an update. *Diabetic Med* 1994; 11: 336-343.
- Chaturvedi N, Stephenson JM, Fuller JH. The relationship between smoking and microvascular complications in the EURODIAB IDDM Complications Study. *Diabetes Care* 1995; 18: 785-792.
- Kullberg CE, Arnqvist HJ. Good blood glucose control characterizes patients without retinopathy after long diabetes duration. *Diabetic Med* 1995; 12: 314-320.
- Couper JJ, Staples AJ, Cocciolone R, Nairn J, Badcock N, Henning P. Relationship of smoking and albuminuria in children with insulin-dependent diabetes. *Diabetic Med* 1994; 11: 666-669.
- De Olivarius F, Andreasen AH, Keiding N, Mogensen CE. Epidemiology of renal involvement in newly-diagnosed middle-aged and elderly diabetic patients. Cross-sectional data from the population-based study 'Diabetes Care in General Practice', Denmark. *Diabetologia* 1993; 36: 1007-1016.
- UK Prospective Diabetes Study Group. UK Prospective Diabetes Study (UKPDS). Urinary albumin excretion over 3 years in diet-treated Type 2 (non-insulin-dependent) diabetic patients, and association with hypertension, hyperglycaemia and hypertriglyceridaemia. *Diabetologia* 1993; 36: 1021-1029.
- Krolewski AS, Laffel LMB, Krolewski M, Quinn M, Warram JH. Glycosylated hemoglobin and the risk of microalbuminuria in patients with insulin-dependent diabetes mellitus. *N Engl J Med* 1995; 332: 1251-1255.
- Almdal T, Feldt-Rasmussen B, Nørgaard K, Deckert T. The predictive value of microalbuminuria in IDDM. *Diabetes Care* 1994; 17: 120-125.
- Jørgens V, Grüsser M, Bött U, Mühlhauser I, Berger M. Effective and safe translation of intensified insulin therapy to general internal medicine departments. *Diabetologia* 1993; 36: 99-105.
- Bött U, Jørgens V, Grüsser M, Bender R, Mühlhauser I, Berger M. Predictors of glycaemic control in Type 1 diabetic patients after participation in an intensified treatment and teaching programme. *Diabetic Medicine* 1994; 11: 362-371.
- Mühlhauser I, Bött U, Overmann H, Wagener W, Bender R, Jørgens V, Berger M. Liberalized diet in patients with type 1 diabetes. *J Int Med* 1995; 237: 591-597.
- Mühlhauser I, Sulzer M, Berger M. Quality assessment of diabetes care according to the recommendations of the St Vincent Declaration: a population-based study in a rural area of Austria. *Diabetologia* 1992; 35: 429-435.
- Sawicki PT, Heinemann L, Berger M. Comparison of methods for determination of microalbuminuria in diabetic patients. *Diabetic Med* 1989; 6: 412-415.
- Kroll P. Augenärztlicher Untersuchungsbogen zur Früherkennung diabetischer Augenerkrankungen. *Der Augenarzt* 1993; 27: 19-28.
- Retinopathy Working Party. A protocol for screening for diabetic retinopathy in Europe. *Diabetic Med* 1991; 8: 263-267.
- Ferris FL, Kassoff A, Bresnick GH, Bailey I. New visual

- acuity charts for clinical research. *Am J Ophthalmol* 1982; 94: 91-96.
17. McCullagh P. Regression models for ordinal data. *J R Statist Soc* 1980; B42: 109-142.
  18. *SAS Procedures Guide for Personal Computers, Version 6 Edition*. Cary, NC: SAS Institute Inc. 1985.
  19. *SAS/STAT Guide for Personal Computers, Version 6 Edition*. Cary, NC: SAS Institute Inc., 1987.
  20. *SAS Technical Report P-200, SAS/STAT Software: CALIS and LOGISTIC Procedures*, Release 6.04. Cary, NC: SAS Institute Inc., 1990.
  21. Andersen PK. Multistate models in survival analysis. A study of nephropathy and mortality in diabetes. *Statist Med* 1988; 7: 661-670.
  22. Marshall G, Jones RH. Multistate models and diabetic retinopathy. *Statist Med* 1995; 14: 1975-1983.