

# A Nonlinear Effect of Hyperglycemia and Current Cigarette Smoking Are Major Determinants of the Onset of Microalbuminuria in Type 1 Diabetes

Laura J. Scott,<sup>1,2,4</sup> James H. Warram,<sup>1,2</sup> Linda S. Hanna,<sup>1</sup> Lori M. B. Laffel,<sup>1,4</sup> Louise Ryan,<sup>3</sup> and Andrzej S. Krolewski<sup>1,2,4</sup>

Cigarette smoking and poor glycemic control are risk factors for diabetic nephropathy in type 1 diabetes. However, the specifics of the relation of these risk factors to the onset of this complication have not been elucidated. To investigate these issues, we followed for 4 years 943 Joslin Clinic patients aged 15–44 years with type 1 diabetes who had normoalbuminuria during the 2-year baseline period. Microalbuminuria developed in 109 of the 943 individuals, giving an incidence rate of 3.3/100 person-years. The risk of onset of microalbuminuria was predicted somewhat more precisely by the measurements during the 1st and 2nd years preceding onset than by all the measurements during the longer (4-year) interval, suggesting attenuation of the impact of past hyperglycemia over time. Point estimates of the incidence rate (per 100 person-years) according to quartiles of HbA<sub>1c</sub> during the 1st and 2nd years preceding the outcome were 1.3 in the 1st, 1.5 in the 2nd, 3.1 in the 3rd, and 6.9 in the 4th ( $P = 1.3 \times 10^{-9}$ ). Point estimates of the incidence rate (per 100 person-years) according to smoking status were 7.9 for current smokers, 1.8 for past smokers, and 2.2 for those who had never smoked ( $P = 2.0 \times 10^{-7}$ ). In a multiple logistic model, the independent effects of HbA<sub>1c</sub> level and cigarette smoking remained highly significant, but their magnitudes were reduced. Using the same covariates in a generalized additive model, we examined the shape of the relationship between HbA<sub>1c</sub> and onset of microalbuminuria and found significant nonlinearity in the logarithm of odds scale ( $P = 0.04$ ). The slope was steeper with HbA<sub>1c</sub> >8% than <8%. Furthermore, the change in the slope was magnified among current smokers. In conclusion, patients with type 1 diabetes who smoke and have an HbA<sub>1c</sub> >8% have the highest risk of onset of microalbuminuria. *Diabetes* 50:2842–2849, 2001

It is estimated that 35% of individuals with type 1 diabetes will develop advanced diabetic nephropathy, although the risk may be declining with time (1–3). Individuals with advanced diabetic nephropathy are at greatly increased risk of cardiovascular disease and death from end-stage renal failure (1,4,5). Identification of the determinants of the onset of early diabetic nephropathy is, therefore, essential for reducing the morbidity and mortality associated with diabetes. After 30 years' duration of illness, about half of type 1 diabetes patients have developed microalbuminuria, the earliest clinically recognized stage of diabetic nephropathy (6,7). Many studies have identified poor glycemic control as a strong risk factor for the onset of microalbuminuria (8–18), but debate continues over the shape of the relation between the level of hyperglycemia and risk of microalbuminuria (10,14,16,19).

The mechanisms underlying this relation are unknown. These include not only the biological nature of the renal injury induced by hyperglycemia, but also how the injury accumulates and what processes exist to repair it. Of great interest, therefore, are studies that attempt to dissect the temporal aspects of renal injury, such as the delay between exposure to an “effective” dose of hyperglycemia and the onset of microalbuminuria (latent period). The findings of these studies may indicate the extent to which the renal injury that results from microalbuminuria is “functional” (in the sense that it occurs after short exposure and is not cumulative) or “morphological” (in the sense that it develops after long exposure and is cumulative).

The evidence for cigarette smoking as a risk factor for diabetic nephropathy in type 1 diabetes has been inconsistent. Many studies have found cigarette smoking to be a significant risk factor for advanced stages of diabetic nephropathy (20–23), but other research has not supported this association (24,25). The discrepancies might have resulted from increased mortality of those who smoked cigarettes. Only a few studies have examined smoking as a risk factor for the onset of microalbuminuria; some have shown significant association between the development of microalbuminuria and smoking (12,26,27), whereas others have not (11,28). In the latter studies, the effect of smoking cessation as a confounding factor and a

From the <sup>1</sup>Section of Genetics and Epidemiology, Research Division, Joslin Diabetes Center, Boston, Massachusetts; the <sup>2</sup>Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts; the <sup>3</sup>Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts; and the <sup>4</sup>Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Address correspondence and reprint requests to Dr. Andrzej S. Krolewski, Genetics and Epidemiology, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215. E-mail: andrzej.krolewski@joslin.harvard.edu.

Current address for L.J.S. is Department of Biostatistics, School of Public Health, University of Michigan, Ann Arbor, MI.

Received for publication 14 January 2001 and accepted in revised form 18 September 2001.

ACR, ratio of albumin and creatinine ratio in urine; DCCT, Diabetes Control and Complications Trial; GAM, generalized additive model.

possible interaction between poor glycemic control and smoking did not receive sufficient attention.

In this study, we examined the individual and combined effects of HbA<sub>1c</sub> levels and smoking on the incidence rate of microalbuminuria in the Natural History of Microalbuminuria Study, a prospective cohort study of patients with type 1 diabetes attending the Joslin Clinic (7,14,29). Within this cohort, >1,000 patients with normoalbuminuria were identified during the 2-year baseline period, and the majority of them have been followed for ≥4 years.

## RESEARCH DESIGN AND METHODS

Study protocols for baseline and follow-up examinations were approved by the Committee on Human Studies at the Joslin Diabetes Center.

**Study participant selection.** Between 1 January 1991 and 31 March 1992, a 50% sample of patients aged 15–44 years attending the Internal Medicine or Pediatric Departments of the Joslin Clinic were screened for microalbuminuria in random urine specimens. Details of patient selection criteria and methods have been published previously (7,14,29), and an abbreviated description is included here. By 31 March 1992, 1,602 patients with type 1 diabetes had been screened at least once for microalbuminuria. During the next 2 years (the baseline interval), whenever they returned to the clinic for their routine care, their random urine specimens were examined for microalbuminuria. After 2 years had elapsed from the date of the first urine specimen, the nephropathy status of each patient at baseline was determined according to a consensus (defined below) of the available urine specimens. The 2-year baseline interval was selected because a majority of patients (61%) had at least three samples tested within that time. Of the 1,602 patients screened, 1,080 had normoalbuminuria. Of these, 943 patients provided follow-up urine specimens as described below. The remaining 137 (13%) provided no follow-up urines and were excluded from this analysis.

**Assessment of urinary albumin excretion.** We used the ratio of urinary concentrations of albumin and creatinine (ACR; expressed in mg/g) for both the diagnosis of normoalbuminuria at baseline and the follow-up assessment of urinary albumin excretion. Urinary albumin concentration was measured by immunonephelometry (Behring, Somerville, NJ), and urinary creatinine concentration was measured by colorimetry (modified Jaffe reaction) on an Astra-7 automated system (Beckman Instruments, Brea, CA). Details of the laboratory methods have been published previously (7). Sex-specific criteria for normal urinary albumin excretion, as assessed by the ACR in a random urine, have been published previously: <17 mg/g for men and <25 mg/g for women (7). Higher values are considered to represent microalbuminuria (or proteinuria if ≥250 mg/g for men and ≥355 mg/g for women). During the initial phase of the study, urine samples with an albumin concentration <10 mg/l were deemed within the normal range and urinary creatinine was not determined (40% of the baseline samples). Subsequently, creatinine was determined in all urine samples regardless of the albumin concentration.

**Consensus classification of nephropathy status.** The diagnosis of normoalbuminuria during the 2-year baseline interval was based on a consensus of the available ACRs in that interval. For those with three or more measurements (61%), we required that >50% of the available specimens be in the normoalbuminuria range. For those with only two measurements (22%), we required that either both were in the normal range or, if one was abnormal, it was close enough to the normal range that the geometric mean of the two was within the normal range. Those with a single measurement (17%) were classified according to that single value.

**Follow-up assessment of outcome.** For 4 years after the baseline period, we continued to measure the ACR in random urine samples collected from members of the cohort whenever they returned to the clinic. The 4-year period was divided for analytical purposes into 2-year intervals, designated as the first and second follow-up intervals. The distribution of patients according to the number of ACR measurements available during each follow-up interval remained fairly stable through both follow-up intervals (49 and 56% with three or more measurements, 25 and 21% with two, and 26 and 23% with one, for the first and second follow-up intervals, respectively). The same consensus criteria for determining a patient's nephropathy status in the baseline interval were applied to the results in each follow-up interval. Persistent microalbuminuria was considered to have developed if the available ACR measurements in a follow-up interval failed to meet the criteria for normoalbuminuria. For patients with three or more measurements, this meant that half or more were abnormal; for those with two measurements, we required that both were abnormal or, if one was normal, it was close enough to abnormal that the geometric mean of the two was in the abnormal range, and for those with a single measurement during the interval, it was in the abnormal range.

Because we required that a diagnosis of microalbuminuria be confirmed in a second urine sample (hence the designation persistent microalbuminuria), we repeated the analyses in this report after excluding the minority of microalbuminuria onsets that were based on a single ACR. The results, which were not materially affected, are summarized in a footnote to Table 3.

**Ghb.** Since 1988, Ghb has been assayed by the Joslin Clinical Laboratory at the time of routine clinic visits. In January 1995, the assay method was changed from an electrophoretic method (Corning Medical and Scientific, Corning, NY) for measuring HbA<sub>1</sub> to an ion-exchange chromatographic method (Variant; Bio-Rad, Hercules, CA) for the subfraction HbA<sub>1c</sub>. Both methods were run together for 4 months, and the correlation coefficient for duplicate determinations on the same specimens was 0.98. The HbA<sub>1</sub> values were converted to HbA<sub>1c</sub> values by the following formula: HbA<sub>1c</sub> = (HbA<sub>1</sub> - 0.14)/1.23 (14).

**Assessment of hyperglycemic exposure.** The temporal relation between exposure to a level of hyperglycemia and the onset of microalbuminuria is unknown. Therefore, to select the best measure of glycemic exposure, we examined the dosage-response relation between the onset of microalbuminuria in each follow-up interval and summary measures of HbA<sub>1c</sub> during three specific time periods preceding that follow-up interval. For this purpose, HbA<sub>1c</sub> values for each study patient were retrieved from the laboratory's archived databases and grouped into the following time periods: 1) 3rd and 4th years preceding each follow-up interval; 2) 1st and 2nd years preceding each follow-up interval; and 3) a weighted average of the first two values representing the period 1st to the 4th years preceding each follow-up interval. The average number of measurements of HbA<sub>1c</sub> within these time periods were 3.5, 4.3, and 7.8, respectively. For all individuals, the geometric mean of their HbA<sub>1c</sub> measurement in each 2-year interval was considered an integrated measure of their exposure to hyperglycemia during that interval. The quartiles of the distributions of the geometric means of the HbA<sub>1c</sub> for the 1st and 2nd and the 3rd and 4th years preceding the follow-up interval were almost identical, and the Spearman correlation was 0.69. As an integrated measure spanning the 4-year time period, the two measures were averaged to weight the contributions of each 2-year interval equally.

**Medical record abstracting.** All of the available medical records at the Joslin Clinic for individuals included in the study were abstracted. Information abstracted separately for baseline and the first follow-up interval included the first three measurements of systolic and diastolic blood pressure and the first measurement of weight in the interval. The mean of the systolic and diastolic blood pressures from each interval were used in the analysis. Height was abstracted from medical record, and BMI was calculated as kg/m<sup>2</sup> for the baseline interval.

**Cigarette smoking.** Information regarding smoking status during the baseline interval was gathered by questionnaire (supplemented by telephone interviews with nonrespondents). Participants were asked if they currently smoked or had smoked in the past and, if so, the ages at which they had started and stopped smoking. Current cigarette smoking was defined as smoking at any point in the baseline interval. Past smoking was defined as smoking at an earlier time but not smoking in the baseline interval. The remainder had never smoked. Smoking status was determined for 75% (*n* = 709) of the cohort. The 25% without information differed from those with smoking information in several ways. They were younger (26 ± 8 vs. 29 ± 8 years; *P* = 0.0001), had shorter duration of diabetes (11 ± 8 vs. 13 ± 8 years; *P* = 0.001), and had lower systolic blood pressure (116 ± 10 vs. 118 ± 12; *P* = 0.001).

**Statistical analysis.** Analyses of univariate and generalized linear models were performed in SAS (SAS 6.12 for Windows; SAS Institute, Cary, NC). Generalized additive models (GAMs) were fit using SPLUS (Version 4.5; StatSci, MathSoft, Seattle, WA). Comparisons of baseline values of continuous and categorical variables among patients with and without onset of microalbuminuria were carried out using *t* tests and  $\chi^2$  tests. Variations in the level of potential confounders by categorized levels of baseline HbA<sub>1c</sub> were assessed using analysis of variance. To estimate the incidence rate of the onset of microalbuminuria in the entire cohort, a person was credited with 2 person-years of follow-up for each interval in which they remained normoalbuminuric, and 1 person-year in the interval in which they had the onset (29).

Univariate and multiple logistic regression analyses were performed using pooled logistic regression (30). Pooled logistic regression is essentially a discrete failure time approach and was suggested by the use of a consensus of multiple ACR measurements obtained over a 2-year interval to define onset of microalbuminuria, as opposed to a single measurement that would have a specific date. This means that individuals entered the logistic regression for follow-up one time from baseline to the first follow-up interval and a second time from the first to second follow-up intervals, provided they did not have an event during the first follow-up interval. One complication was the group of 76 individuals who had no ACR measurement in the first follow-up interval but

TABLE 1

Baseline characteristics of the study group with type 1 diabetes and normoalbuminuria according to whether or not microalbuminuria developed during follow-up

Characteristic at baseline	Normoalbuminuria remained ( <i>n</i> = 834)	Microalbuminuria developed ( <i>n</i> = 109)	<i>P</i>
Age at diagnosis of diabetes (years)	16.3 ± 8.5	14.3 ± 8.6	0.02
Duration of diabetes (years)	12.4 ± 8.4	14.3 ± 8.4	0.03
Current age (years)	28.7 ± 7.8	28.6 ± 8.0	0.81
HbA <sub>1c</sub> (%)	8.1 ± 1.4	9.1 ± 1.6	0.0001
SBP (mmHg)	117.5 ± 11.7	119.4 ± 12.7	0.16
DBP (mmHg)	72.7 ± 7.6	73.6 ± 7.2	0.23
BMI (kg/m <sup>2</sup> )	24.3 ± 3.0	24.1 ± 3.2	0.72
Women (%)	54	53	0.85
Smoking status (%)			
Current	21	48	0.001
Past	16	9	
Never	63	43	

Data are means ± SD or %. *P* value from *t* or  $\chi^2$  test. DBP, diastolic blood pressure; SBP, systolic blood pressure.

did have measurements in the second follow-up interval. Random imputation was used to assign an interval to events that occurred in that group (31).

The model for multiple logistic regression analyses included as covariates HbA<sub>1c</sub>, smoking status, age at diagnosis of diabetes, and duration of diabetes (32). As a consequence of the study design, all models also included an indicator variable for the follow-up interval of analysis (first or second). Baseline smoking status was used for both periods of analysis. Because of missing creatinine values for many baseline urine samples that had very low albumin levels (<10 mg/l; see above), models that included ACR as a covariate were run in two ways. The first method restricted the analysis to the second follow-up interval for which urine creatinine as well as albumin were measured for all urine samples. The second method replaced each missing baseline ACR value with an average ACR based on urine samples with the same albumin concentration that did have creatinine measured. The resulting odds ratios were similar. Only the later analysis with its much larger sample size is presented.

We used GAMs, within the discrete failure time approach, to assess the linearity (in the logarithm of odds scale) of the relationship between HbA<sub>1c</sub> and onset of microalbuminuria. A GAM allows for relaxation of the assumption of linearity by using a flexible smoothing term—in this case the loess option—to fit a smooth curve to the ungrouped data (33). We examined a variety of spans and found that a span of 0.7 best represented the association, as judged by Akaike's information criterion. The results are presented as predicted incidence rates, with the shaded region representing the pointwise ± 2 SE band. Interactions between HbA<sub>1c</sub> and smoking status (current smokers versus past smokers/those who never smoked) were tested using linear and nonlinear terms for HbA<sub>1c</sub>.

For calculation of adjusted incidence rates within various subgroups of patient characteristics, the confounding variables were set to their mean or observed proportions. The 2-year follow-up intervals were taken into account in estimating the risk of onset per year and the incidence rate per 100 person-years from the logistic and GAM models, respectively (32).

## RESULTS

The cohort of eligible individuals for this study consisted of 1,080 Massachusetts residents ages 15–44 years with type 1 diabetes (94% of them Caucasian) who were classified as normoalbuminuric during the 2-year baseline interval. The average age at diagnosis of type 1 diabetes in the cohort was 15 ± 8 years, and the average duration of diabetes at baseline was 13 ± 8 years. Follow-up screening for microalbuminuria was obtained from 943 individuals (87%). Of those followed, 92% were screened in the first 2-year follow-up interval and 84% in the second 2-year follow-up interval, with a resulting 3,245 person-years of follow-up. Persistent microalbuminuria (hereafter referred to as microalbuminuria) developed in 109 individuals in either the first or second follow-up interval, giving an

incidence rate of microalbuminuria of 3.3 per 100 person-years.

Baseline characteristics of the patients are summarized in Table 1 according to whether microalbuminuria developed during follow-up. Compared to those remaining normoalbuminuric, the patients with the onset of microalbuminuria had a longer duration of diabetes (2 years on average; *P* = 0.03) and a correspondingly younger age at diagnosis of diabetes (*P* = 0.02). The strongest associations were a higher HbA<sub>1c</sub> in those with the onset of microalbuminuria (9.1 vs. 8.1%; *P* = 0.0001) and a higher prevalence of current cigarette smokers (48 vs. 21%; *P* = 0.001). Current age, blood pressure, BMI, and sex were similar in the two groups.

An important aim of this study was to explore the temporal relationship between exposure to a level of hyperglycemia, as reflected in HbA<sub>1c</sub> levels, and the risk of onset of microalbuminuria. Toward that end, we examined three summary measures of HbA<sub>1c</sub> that had different temporal relationships with each follow-up interval. The first measure was the geometric mean of HbA<sub>1c</sub> values obtained during the 3rd and 4th year preceding each follow-up interval. The second measure was the geometric mean of HbA<sub>1c</sub> values obtained during the 1st and 2nd year preceding each follow-up interval. The third measure was the average of the first two measures, and represents an integrated measure of the glycemic exposure during the 1st, 2nd, 3rd, and 4th years preceding each follow-up interval. Each measure of HbA<sub>1c</sub> was divided approximately into quartiles using the ranges 4.3–7.2, 7.3–8.1, 8.2–9.0, and 9.1–16.5%, which were based on the distribution of HbA<sub>1c</sub> values during the initial baseline interval. Incidence rates of microalbuminuria were estimated for each quartile (Table 2).

The HbA<sub>1c</sub> measure based on values obtained during the 3rd and 4th years preceding each follow-up interval did not separate the study group very well into a gradient of risk levels for the onset of microalbuminuria (Table 2). The incidence rates for the first three quartiles were identical, and only the highest quartile had an elevated incidence rate—about three times as high as the lower quartiles. The HbA<sub>1c</sub> measure based on values obtained during the 1st

TABLE 2

Incidence rate of microalbuminuria (per 100 person-years) according to HbA<sub>1c</sub> value during different intervals preceding follow-up (different windows of exposure)

Interval preceding follow-up	HbA <sub>1c</sub> value (%)				Onsets (n)	Person-Years (n)	$\chi^2$	P
	4.3–7.2	7.3–8.1	8.2–9.0	9.1–16.5				
1st and 2nd years	1.3	1.5	3.1	6.9	99	3,013	55.8	$1.3 \times 10^{-9}$
3rd and 4th years	2.2	2.2	2.2	6.4	102	3,082	34.9	$4.7 \times 10^{-6}$
1st to 4th years	1.4	1.7	3.0	6.5	92	2,886	44.8	$1.6 \times 10^{-7}$

Data are percentages or *n*. Detailed definitions of the intervals (exposure windows) are included in RESEARCH DESIGN AND METHODS. Ranges shown for HbA<sub>1c</sub> values are the quartiles of the distribution of HbA<sub>1c</sub> values during baseline (see Table 1). The quartile ranges of HbA<sub>1c</sub> were almost identical in all three windows. Numbers for onsets and person-years vary because of missing data on HbA<sub>1c</sub> during one or both 2-year intervals for a few patients. When all three models were restricted to the subgroup with HbA<sub>1c</sub> values for both intervals, *P* values were  $1.0 \times 10^{-8}$ ,  $1.3 \times 10^{-6}$ , and  $1.6 \times 10^{-7}$ .

and 2nd years preceding each follow-up interval gave a slightly higher incidence rate for the highest quartile (6.9 vs. 6.4 per 100 person-years) and a lower incidence rate for the lowest quartile (1.3 vs. 2.2 per 100 person-years) (Table 2). Therefore, HbA<sub>1c</sub> values during the 1st and 2nd years appear to be better discriminators of risk than the HbA<sub>1c</sub> measure during the 3rd and 4th years before follow-up.

When the two HbA<sub>1c</sub> measures were combined to give a measure based on a larger number of HbA<sub>1c</sub> determinations that spanned 4 instead of 2 years, the incidence rates were not better than those based on only the 1st and 2nd years before follow-up. Addition of the HbA<sub>1c</sub> values from the earlier time period produced a slight dampening of the risk gradient rather than an accentuation of it, as would be expected if the effects of hyperglycemia were cumulative over the whole 4-year period. To compare the measurements directly, we restricted the analysis to people with data in both 2-year intervals. As judged by  $\chi^2$  values for the coefficients for the HbA<sub>1c</sub> variables and Akaike's information criterion, the model using just the most recent HbA<sub>1c</sub> values was a better discriminator of the risk of microalbuminuria than the measure based on 4 years (see footnote to Table 2). Accordingly, in all subsequent analyses we used the geometric mean of the HbA<sub>1c</sub> values during the 1st and 2nd years preceding the follow-up interval.

In preparation for evaluating the dosage-response relation between hyperglycemia and the risk of onset of microalbuminuria without the potentially confounding effects of other risk factors, we examined the association of the significant risk factors identified in Table 1 with the level of HbA<sub>1c</sub>. Patients in the lowest quartile of baseline HbA<sub>1c</sub> were, on average, 3 years older at the diagnosis of diabetes and had a duration of diabetes 2 years shorter than patients in the higher quartiles. Of greater importance was that the proportion currently smoking increased progressively from 14% in the 1st quartile to 41% in the 4th quartile of HbA<sub>1c</sub>.

Current smoking, therefore, was associated with both the onset of microalbuminuria and the level of HbA<sub>1c</sub>, so it is a confounder of univariate estimates of the effect of HbA<sub>1c</sub> on the risk onset of microalbuminuria. To evaluate the independent effect of HbA<sub>1c</sub> level, we used multiple logistic analysis of these variables in patients with information on both smoking and HbA<sub>1c</sub> (Table 3). Adjustment for smoking, age at diagnosis of diabetes, and duration of diabetes reduced the odds ratio for the highest quartile of HbA<sub>1c</sub> from 6.3 in the unadjusted analysis to 4.7 (a 25% reduction). Inclusion of smoking in the model was respon-

sible for most of the decrease in the estimates of the effect of HbA<sub>1c</sub>.

To examine the shape of the association between HbA<sub>1c</sub> and onset of microalbuminuria in more detail, we used a GAM that allows relaxation of the assumption of linearity between HbA<sub>1c</sub> exposure and the outcome (24). The model was adjusted for smoking, age at diagnosis of diabetes, and duration of diabetes. There was a significant nonlinear component to the fit (*P* = 0.04): the incidence rate of onset was relatively constant for HbA<sub>1c</sub> values of ~8% and then increased for HbA<sub>1c</sub> values >8% (Fig. 1). The change in the slope between the lower and upper ranges of HbA<sub>1c</sub> indicated that the effect of a proportionate difference in HbA<sub>1c</sub> depended on whether the HbA<sub>1c</sub> was above or below 8%. To illustrate this finding, we estimated incidence rates in two distinct parts of the curve: at HbA<sub>1c</sub> values 7.2 and 8% in the lower range and 9 and 10% in the upper range. For each pair of HbA<sub>1c</sub> values, the lower was 10% less than the higher. The incidence rates for HbA<sub>1c</sub>

TABLE 3

Multiple logistic analysis of the onset of microalbuminuria according to quartile of HbA<sub>1c</sub> and smoking status

Variable	Model*	
	Univariate odds ratios	Adjusted odds ratios
HbA <sub>1c</sub> (%)		
4.3–7.2	1 (reference)	1 (reference)
7.3–8.1	0.98 (0.4–2.4)	0.85 (0.3–2.1)
8.2–9.0	2.3 (1.1–5.0)	1.9 (0.9–4.2)
9.1–16.5	6.3 (3.1–12.7)	4.7 (2.3–9.7)
Smoking status		
Never	1 (reference)	1 (reference)
Past	0.84 (0.4–1.8)	0.81 (0.4–1.8)
Current	3.8 (2.4–6.0)	3.1 (1.9–5.1)

Data are *n* (95% CI) and include only patients with HbA<sub>1c</sub> and smoking information. (This includes 80 out of 109 of those with onset of microalbuminuria and 1,183 2-year follow-up intervals for analysis.) Univariate model was adjusted for period of analysis only. Adjusted model included both the quartiles of HbA<sub>1c</sub> and smoking status, and is adjusted for age at diabetes diagnosis, duration of diabetes, and period of analysis. When the analysis was repeated using only individuals with three or more ACR measurements (or two concordant measurements) in both the baseline and follow-up interval (784 2-year follow-up intervals and 44 onsets of microalbuminuria), the results were unchanged. The odds ratios were 0.79, 1.9, and 4.4 for the second, third, and fourth quartiles of HbA<sub>1c</sub> relative to the risk of microalbuminuria in the first quartile. The odds ratio for current smokers was 3.5 and for past smokers was 0.31 relative to the risk of microalbuminuria for those who never smoked.

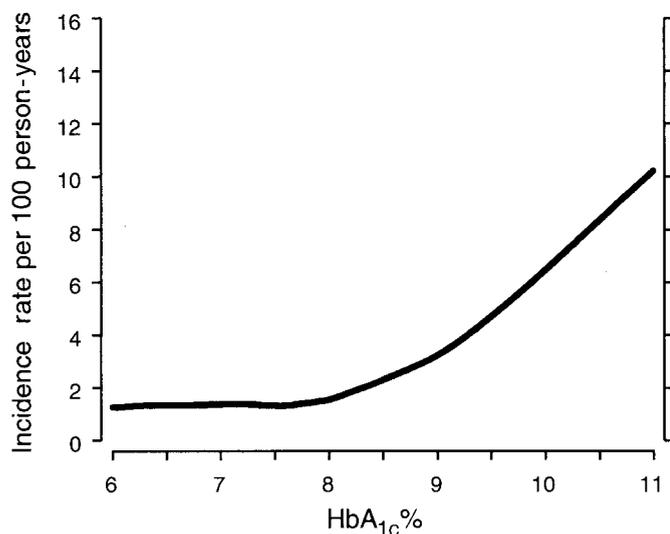


FIG. 1. Predicted incidence rate of the onset of microalbuminuria according to HbA<sub>1c</sub>. The value used for HbA<sub>1c</sub> is the geometric mean of the measurements obtained during the 2-year interval preceding the outcome. Using a discrete failure time approach, a GAM was constructed that fitted a smooth curve to the ungrouped data using the loess option with a span = 0.7 (see RESEARCH DESIGN AND METHODS). The model adjusted for smoking, duration of diabetes, and age at diagnosis of diabetes. The shaded region represents the pointwise  $\pm 2$  SE band.

values at 7.2 and 8% were 1.36 and 1.54 per 100 person-years, respectively. This gave a difference of 0.18 onsets per 100 person-years. In contrast, the incidence rates for HbA<sub>1c</sub> 1 and 10% were 3.24 and 6.53 per 100 person-years, respectively. This gave a difference of 3.29 onsets per 100 person-years. These rate differences correspond to rate reductions of 12% (from 1.54 to 1.36) in the lower range and 50% (from 6.53 to 3.24) in the upper range.

The effect of smoking on risk of onset of microalbuminuria was evaluated in a similar way. In the univariate analysis shown in Table 3, current smokers had a substantially elevated relative odds in comparison with those who never smoked (3.3; 95% CI 2.1–5.2), whereas past smokers did not (0.82; 95% CI 0.39–1.7). The incidence rate of onset of microalbuminuria (per 100 person-years) was 7.9 for current smokers, 1.8 for past smokers, and 2.2 for never smokers ( $P = 2.0 \times 10^{-7}$ ). To evaluate the independent effect of smoking, we used the multiple logistic analysis summarized in Table 3. Adjustment for HbA<sub>1c</sub> and other potential confounders reduced the odds ratio for current smoking from 3.8 in the univariate analysis to 3.1 (an 18% reduction). The decrease was mainly attributable to the inclusion of HbA<sub>1c</sub> in the model. Interestingly, when we restricted our analysis to individuals with at least three ACR measurements (or two concordant measurements) in both the baseline and the follow-up interval, the adjusted odds ratios for the 2nd through 4th quartiles of HbA<sub>1c</sub> relative to the 1st were unchanged from the results of the original analysis (see legend to Table 3). The same was true for the current smokers.

To examine the joint effects of cigarette smoking and HbA<sub>1c</sub> on the risk of onset of microalbuminuria, we fitted a GAM to obtain predicted incidence rates stratified by smoking status (Fig. 2). The model included an interaction term between the effects of smoking and HbA<sub>1c</sub> and, as before, was adjusted for age at diagnosis of diabetes and

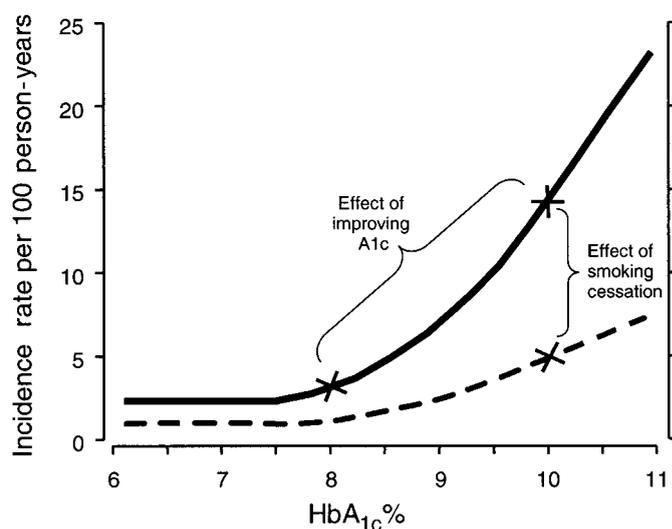


FIG. 2. Predicted incidence rate of the onset of microalbuminuria according to the level of HbA<sub>1c</sub>, estimated separately for current cigarette smokers ([solid line]) and nonsmokers ([broken line]). The brackets illustrate the possible benefits that cigarette smokers with an HbA<sub>1c</sub> = 10% might gain either from improving HbA<sub>1c</sub> to 8% or from ceasing to smoke. The HbA<sub>1c</sub> value shown is the geometric mean of the measurements obtained during the 2-year interval preceding the outcome. Using a discrete failure time approach, a GAM was constructed that fitted a smooth curve using the loess option with span = 0.7 (see RESEARCH DESIGN AND METHODS). The model adjusted for duration of diabetes and age at diagnosis of diabetes.

duration of diabetes. The estimated incidence rates for nonsmokers and smokers at an HbA<sub>1c</sub> of 8% were 1.3 and 4.8 per 100 person-years, respectively, a difference of 3.5 onsets per 100 person-years for smoking. The estimated incidence rates at HbA<sub>1c</sub> values 8 and 10% among nonsmokers were 1.3 and 3.4 per 100 person-years, respectively, a difference of 2.1 onsets per 100 person-years for the effect of the higher HbA<sub>1c</sub>. Neither of the differences associated with a single exposure were very large, but the presence of both factors together was accompanied by a very high estimated incidence rate—15.0 onsets per 100 person-years. The risk associated with exposure to both factors was more than additive. When either was evaluated as a second exposure, it was associated with a much larger effect than it had as a single exposure. Among individuals with HbA<sub>1c</sub> of 10%, the added risk for smokers was 11.6 onsets per 100 person-years. Alternatively, among smokers, the added risk for those with poor glycemic control (HbA<sub>1c</sub> = 10%) was 10.2 onsets per 100 person-years. With due allowance for this being an observational study, these differences could be viewed as indications of the potential benefits of either smoking cessation or improved glycemic control among smokers with poor glycemic control (Fig. 2).

The baseline level of the ACR has often been included in models of the risk of microalbuminuria (8,12,15,18,28). Interpretation of the estimated effect of this variable is problematic for both biological and statistical reasons, as ACR levels are also the outcome of interest (34). Nonetheless, ACR is a potentially useful clinical predictor of microalbuminuria. To test our findings while simultaneously taking baseline ACR into consideration, we included it in the multiple logistic model that included HbA<sub>1c</sub>, smoking, and other potential confounders. A dou-

bling of baseline ACR increased the odds by a factor of 4.1 (95% CI 2.9–5.9). Estimates of the odds ratios for HbA<sub>1c</sub> and current smoking in this model were reduced only slightly (15 and 5%, respectively), indicating that their effects were independent of baseline ACR.

## DISCUSSION

We followed prospectively for 4 years a large cohort of patients with type 1 diabetes to elucidate the association of two risk factors, hyperglycemia and cigarette smoking, to the risk of onset of microalbuminuria. Particular attention was given to the relation between onset of microalbuminuria and HbA<sub>1c</sub> levels at various times in the past.

We found that the risk of onset of persistent microalbuminuria in each 2-year follow-up interval was more closely related to the HbA<sub>1c</sub> measurements during the preceding 2-year study interval than to the interval 3–4 years prior or the average over the whole 4-year interval. If the renal injury caused by hyperglycemia is retained over a long time, the accumulated injury would be more closely reflected in the HbA<sub>1c</sub> values from the preceding 4 years than in the values from just the preceding 2 years. Therefore, our results suggest that, although exposure to significant hyperglycemia even for a period of 2 years increases the risk of microalbuminuria during the subsequent 2 years, this effect is subsequently attenuated. One possibility is that, in part, the renal injury induced by hyperglycemia is functional and not carried over from earlier exposures, although more long-term data are needed to evaluate the extent and speed with which the effect of a period of severe hyperglycemia is attenuated.

This finding is consistent with the results of the Diabetes Control and Complications Trial (DCCT) study (10). In the primary as well as in the secondary intervention cohorts, the effect of intensive diabetes treatment on reducing the risk of microalbuminuria was observed only after good glycemic control was maintained for 3 years. Curiously, the recently published results of the post-trial follow-up appear at first glance to be inconsistent with this observation, in that the difference in risk in the two treatment groups has persisted despite the convergence of their HbA<sub>1c</sub> distributions (35). Microalbuminuria had developed in 11% of those formerly in the conventional treatment group (average HbA<sub>1c</sub> 9.1%) and in 5% of those formerly in the intensive treatment group (average HbA<sub>1c</sub> 7.1%) (35). It should be noted, however, that in this post-trial study, significant differences in HbA<sub>1c</sub> levels between the two groups persisted during the first 3 years and became insignificant only during the 4th year. Furthermore, microalbuminuria was evaluated in a single specimen taken 3.5 years after the end of the trial, and it is unknown how soon after the trial the microalbuminuria developed or in what proportion of these cases the microalbuminuria was transient rather than sustained. Our results suggest that the higher risk of onset of microalbuminuria in the conventional treatment group would persist 2 years beyond the end of the trial and perhaps longer, taking into account the persisting differences in HbA<sub>1c</sub> between the study groups. Only subsequently would the difference between the groups decrease in response to the convergence of their HbA<sub>1c</sub> distributions during the first several years of post-trial follow-up.

The results of our study and the DCCT are evidence that the risk of onset of persistent microalbuminuria is substantially determined by the level of hyperglycemia during a 2- to 3-year interval (window of exposure) preceding the onset of microalbuminuria. This has implications for further studies as well as for patient care. The use of a baseline value (that becomes increasingly remote during follow-up) or a cumulative average of measurements (that includes remote values no longer relevant to the current risk) may not give an accurate representation of the dosage-response relationship. This conclusion, however, must be qualified, because no one has specifically examined the effect of more remote exposures.

The shape of the dosage-response relationship between levels of HbA<sub>1c</sub> and the risk of onset of microalbuminuria in the current study is similar to that obtained in our previous cross-sectional study (14). The shift to a steeper slope in the upper ranges of HbA<sub>1c</sub> indicates that the effect of a proportionate difference in HbA<sub>1c</sub> depends on whether the HbA<sub>1c</sub> is above or below 8%. In the illustration given in RESULTS, a 10% decrease in HbA<sub>1c</sub> from 8 to 7.2% was associated with a 12% lower risk of onset, whereas a 10% decrease in HbA<sub>1c</sub> from 10 to 9% was associated with a 50% lower risk of onset. From this, one may infer that the expected benefit of improved glycemic control will depend in a similar way on whether the starting point is above or below HbA<sub>1c</sub> = 8%, as well as an individual's constellation of other risk factors (such as cigarette smoking, as suggested by the data in Fig. 2).

In contrast, according to the dosage-response relationship modeled by DCCT investigators for "sustained microalbuminuria," the expected risk reduction from a 10% improvement in HbA<sub>1c</sub> is virtually the same in the ranges below and above 8.0, 49, and 35% (19). Two possible reasons for the different results from the DCCT and our study should be considered. (It should be noted that our comparisons between the two studies are restricted to DCCT results for "sustained microalbuminuria" [based on two measurements] because it is closer to our end point [based on two or more measurements for 71% of cases] than their less reliable end point of "microalbuminuria" [based on one measurement] [19]).

First, the distribution of duration of diabetes was quite different in the two studies. As reported in the DCCT and by us, duration has an impact on the risk of microalbuminuria (7,10). It may also have an impact on the shape of the dosage-response relationship with HbA<sub>1c</sub>. Although there is not enough power in our study to test differences in the shape according to duration, the magnitude of the change in slope at HbA<sub>1c</sub> = 8% was greater for patients with diabetes duration ≥10 years than it was in those with duration <10 years (data not shown). Patients with ≥10 years duration constituted 57% of our study cohort, but only 19% of the DCCT cohort (28).

Second, the two studies used different measures of exposure to hyperglycemia. In our study, we evaluated several indexes of hyperglycemia defined by various exposure windows. We found the strongest association with the geometric mean HbA<sub>1c</sub> during the exposure window 2 years preceding the follow-up interval in which the outcome was evaluated. In the DCCT, the onset of microalbuminuria was related to the mean of all quarterly

measurements of HbA<sub>1c</sub>, up to the annual examination at which the first abnormal test was obtained (10,19,28). Therefore, the exposure windows related to the annual examinations ranged from 1 to 9 years and averaged about 4 years. If one takes as valid our finding that the risk of microalbuminuria is more closely related to the most recent 2 years of exposure than to past exposures, then inclusion of the more remote HbA<sub>1c</sub> values in the updated mean may have blurred the relationship between HbA<sub>1c</sub> and risk in the DCCT study.

In addition to the HbA<sub>1c</sub> level, current smoking was significantly associated with the risk of the onset of microalbuminuria in the current study. Although this finding is consistent with the results of several previous studies (12,26,27), we were able to obtain new detailed information regarding this association. Because smokers had poorer glycemic control than nonsmokers, we examined the effect of smoking after controlling for HbA<sub>1c</sub> using logistic regression models and GAMs. In the former, the effect of smoking was reduced because of its association with poor glycemic control, but the odds ratio remained high and was highly significant statistically. In the GAMs, on the other hand, we demonstrated that the effect of smoking was enhanced in individuals with poor glycemic control. These results indicate that hyperglycemia (as reflected in a high HbA<sub>1c</sub> value) and current smoking may impact the risk of microalbuminuria through different pathophysiological mechanisms.

Because this was an observational study, one must consider the possibility that a person's psychological makeup could predispose her or him to an unhealthy lifestyle that introduces various risk factors for microalbuminuria besides cigarette smoking and poor glycemic control. Therefore, some of the effects attributed to these two factors in our analysis may have been attributable to unmeasured risk behaviors. However, a large effect attributable to unmeasured risk behaviors seems unlikely, as the risk for past smokers was similar to that for those who never smoked. Furthermore, this result may be interpreted as evidence that the smoking-related renal injury is functional rather than cumulative (36). If true, a risk reduction from smoking cessation would be immediate.

Based on our evidence that the effect of smoking on the risk of microalbuminuria is magnified in patients with poor glycemic control, it is not surprising that the DCCT investigators found almost identical risks for current smokers and nonsmokers in the intensive treatment group (2.7 and 2.9 onsets of microalbuminuria per 100 person-years, respectively) (28). In the conventional treatment group, which had an HbA<sub>1c</sub> distribution similar to that of our cohort, the risk for smokers was slightly higher (but not significantly) than in nonsmokers (5.3 and 4.2 per 100 person-years, respectively) (28). This difference is small in comparison to that seen in our study. However, if many of the smokers in the conventional treatment group stopped smoking during the trial, their excess risk would have been diminished, as suggested by the results for past smokers in our study.

Baseline ACR is a strong predictor of the onset of microalbuminuria, as reported previously (8,12,15,18,28). Interestingly, estimates of the effects of current smoking and HbA<sub>1c</sub> were unaffected by inclusion of baseline ACR in

the model—an indication that their effects are independent of ACR's association with the risk of onset. Thus, ACR values within the normal range may be related to genetic determinants of susceptibility to kidney disease rather than to a response to environmental exposures (37). We did not find any association between systolic or diastolic blood pressure and the onset of microalbuminuria. This negative finding must be tempered by our use of measurements made in the clinic, the inaccuracies of which may have diminished our power to detect association. Previous studies, however, have not found a consistent relationship between blood pressure and the risk of microalbuminuria (11,12,15,18,28).

In conclusion, we investigated the relationship of two exposures, cigarette smoking and hyperglycemia (HbA<sub>1c</sub>), to the risk of onset of microalbuminuria. The incidence rate of microalbuminuria changes almost imperceptibly as HbA<sub>1c</sub> increases up to 8% and then rises steeply as HbA<sub>1c</sub> increases beyond 8%. The precise value where the slope changes has not been determined. Current (but not past) smoking increased the risk of microalbuminuria. The effect of the two exposures together is greater than the sum of their separate effects, suggesting some type of interaction. One implication from these results is that patients with type 1 diabetes who both smoke and have high HbA<sub>1c</sub> levels could greatly benefit from eliminating at least one of the two risk factors.

#### ACKNOWLEDGMENTS

This research was supported by National Institutes of Health (NIH) Grant RO1-DK41526 and the Joslin Diabetes Center. L.J.S. was supported by NIH training grant T32 DK07260-21/22.

We are indebted to the patients of the Joslin Clinic and the staff of the Joslin Diabetes Center, particularly the Reception Desk, Clinical Laboratory, Management Information Systems, and Medical Records Departments for their assistance and cooperation in conducting this study, and to the following members of the Section of Genetics and Epidemiology: F. Denry, M. Davidson, M. Pezzolesi, M. O'Keefe, K. Anderson, D. Butler, N. Castronuovo, M. Wantman, and E. Hart.

#### REFERENCES

- Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T: Diabetic nephropathy in type 1 (insulin-dependent) diabetes: an epidemiological study. *Diabetologia* 25:496–501, 1983
- Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR: The changing natural history of nephropathy in type 1 diabetes. *Am J Med* 78:785–794, 1985
- Kofoed-Enevoldsen A, Borch-Johnsen K, Kreiner S, Nerup J, Deckert T: Declining incidence of persistent proteinuria in type 1 (insulin-dependent) diabetic patients in Denmark. *Diabetes* 36:205–209, 1987
- Krolewski AS, Kosinski EJ, Warram JH, Leland OS, Busick EJ, Asmal AC, Rand LI, Christlieb AR, Bradley RF, Kahn CR: Magnitude and determinants of coronary artery disease in juvenile-onset, insulin-dependent diabetes mellitus. *Am J Cardiol* 59:750–755, 1987
- Jensen T, Borch-Johnsen K, Kofoed-Enevoldsen A, Deckert T: Coronary heart disease in young type 1 (insulin-dependent) diabetic patients with and without diabetic nephropathy: incidence and risk factors. *Diabetologia* 30:144–148, 1987
- Mogensen CE, Christensen CK, Vittinghus E: The stages in diabetic renal disease with emphasis on the stage of incipient diabetic nephropathy. *Diabetes* 32 (Suppl. 2):S64–S78, 1983
- Warram JH, Gearin G, Laffel L, Krolewski AS: Effect of duration of type 1

- diabetes on the prevalence of stages of diabetic nephropathy defined by urinary albumin/creatinine ratio. *J Am Soc Nephrol* 7:930-937, 1996
8. Watts GF, Harris R, Shaw KM: The determinants of early nephropathy in insulin-dependent diabetes mellitus: a prospective study based on the urinary excretion of albumin. *QJM* 79:365-378, 1991
  9. Reichard P, Nilsson BY, Rosenqvist U: The effect of long-term intensified insulin treatment on the development of microvascular complications of diabetes mellitus. *N Engl J Med* 329:304-309, 1993
  10. Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977-986, 1993
  11. Coonrod BA, Ellis D, Becker DJ, Clareann H, Kelsey SF, Lloyd CE, Drash AL, Kuller LH, Orchard TJ: Predictors of microalbuminuria in individuals with IDDM: Pittsburgh Epidemiology of Diabetes Complications study. *Diabetes Care* 16:1376-1383, 1993
  12. Microalbuminuria Collaborative Study Group: Risk factors for development of microalbuminuria in insulin dependent diabetic patients: a cohort study. *BMJ* 306:1235-1239, 1993
  13. Powrie JK, Watts GF, Ingham JN, Taub NA, Talmud PJ, Shaw KM: Role of glycaemic control in the development of microalbuminuria in patients with insulin dependent diabetes. *BMJ* 309:1608-1612, 1994
  14. 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* 332:1251-1255, 1995
  15. Mathiesen ER, Rønn B, Storm B, Foght H, Deckert T: The natural course of microalbuminuria in insulin-dependent diabetes: a 10 year prospective study. *Diabet Med* 12:482-487, 1995
  16. Orchard TJ, Forrest KY, Ellis D, Becker DJ: Cumulative glycaemic exposure and microvascular complications in insulin-dependent diabetes mellitus: the glycaemic threshold revisited. *Arch Intern Med* 157:1851-1856, 1997
  17. Jones CA, Leese GP, Kerr S, Bestwick K, Isherwood DI, Vora JP, Hughes DA, Smith C: Development and progression of microalbuminuria in a clinic sample of patients with insulin dependent diabetes mellitus. *Arch Dis Child* 78:518-523, 1998
  18. Microalbuminuria Collaborative Study Group UK: Predictors of the development of microalbuminuria in patients with type 1 diabetes mellitus: a seven-year prospective study. *Diabet Med* 16:918-925, 1999
  19. Diabetes Control and Complications Trial Research Group: The absence of a glycaemic threshold for the development of long-term complications: the perspective of the Diabetes Control and Complications Trial. *Diabetes* 45:1289-1298, 1996
  20. Christiansen JS: Cigarette smoking and prevalence of microangiopathy in juvenile-onset insulin-dependent diabetes mellitus. *Diabetes Care* 1:146-49, 1978
  21. Muhlhauser I, Sawicki P, Berger M: Cigarette smoking as a risk factor for macroproteinuria and proliferative retinopathy in type 1 (insulin-dependent) diabetes. *Diabetologia* 29:500-502, 1986
  22. Sawicki PT, Didjurgeit U, Muhlhauser I, Bender R, Heinemann L, Berger M: Smoking is associated with progression of diabetic nephropathy. *Diabetes Care* 17:126-131, 1994
  23. Muhlhauser I, Bender R, Bott U, Jörgens V, Grüsser M, Wagener W, Overmann H, Berger M: Cigarette smoking and progression of retinopathy and nephropathy in type 1 diabetes. *Diabet Med* 13:536-543, 1996
  24. West KM, Ahuja MM, Bennett PH, Grab B, Grabauskas V, deCosta MO, Fuller JH, Jarrett RJ, Keen H, Kosaka K, Krolewski AS, Miki E, Schliack V, Teuscher A: Interrelationships of microangiopathy, plasma glucose and other risk factors in 3583 diabetic patients: a multinational study. *Diabetologia* 22:412-420, 1982
  25. Klein R, Klein BE, Moss SE, Cruickshanks KJ: Ten-year incidence of gross proteinuria in people with diabetes. *Diabetes* 44:916-923, 1995
  26. Chase HP, Garg SK, Marshall G, Berg CL, Harris S, Jackson WE, Hamman RE: Cigarette smoking increases the risk of albuminuria among subjects with type 1 diabetes. *JAMA* 265:614-617, 1991
  27. Chaturvedi N, Stephenson JM, Fuller JH: The relationship between smoking and microvascular complications in the EURODIAB IDDM Complications Study. *Diabetes Care* 18:785-792, 1995
  28. DCCT Research Group: Effect of intensive therapy on the development and progression of diabetic nephropathy in the diabetes control and complications trial. *Kidney Int* 47:1703-1720, 1995
  29. Warram JH, Scott LJ, Hanna LS, Wantman M, Cohen SE, Laffel LMB, Ryan L, Krolewski AS: Progression of microalbuminuria to proteinuria in type 1 diabetes. *Diabetes* 49:94-100, 2000
  30. Cox DR, Oakes D: *Analysis of Survival Data*. New York, Chapman & Hall, 1984, p. 101-104
  31. Rubin DB, Schenker N: Multiple imputation in health-care databases: an overview and applications. *Stat Med* 10:585-598, 1991
  32. Greenland S: Introduction to regression modeling. In *Modern Epidemiology*. 2nd ed. Rothman KJ, Greenland S, Eds. Philadelphia, Lipincott-Raven, 1998, p. 417
  33. Hastie TJ, Tibshirani RJ: *Generalized Additive Models*. London, Chapman & Hall, 1990, p. 9-35
  34. Greenland S, Neutra RR: Control of confounding in the assessment of medical technology. *Int J Epidemiol* 9:361-367, 1980
  35. Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions of Complications Research Group: Retinopathy and nephropathy in patients with type 1 diabetes four years after a trial of intensive therapy. *N Engl J Med* 342:381-389, 2000
  36. Ritz E, Ogata H, Orth SR: Smoking: a factor promoting onset and progression of diabetic nephropathy. *Diabetes Metab* 26:54-63, 2000
  37. Fogarty DG, Hanna LS, Wantman M, Warram JH, Krolewski AS, Rich SS: Segregation analysis of urinary albumin excretion in families with type 2 diabetes. *Diabetes* 49:1057-1063, 2000