

# Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial

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## Effect of intensive therapy on the development and progression of diabetic nephropathy in the Diabetes Control and Complications Trial.

The Diabetes Control and Complications Trial (DCCT) has demonstrated that intensive diabetes treatment delays the onset and slows the progression of retinopathy, nephropathy, and neuropathy in patients with IDDM. A detailed description of the effects of this treatment on diabetic nephropathy is presented here. In the primary prevention cohort, intensive treatment reduced the mean adjusted risk of the cumulative incidence of microalbuminuria ( $\geq 28 \mu\text{g}/\text{min}$ ) by 34% (95% CI 2, 56%;  $P = 0.04$ ). Furthermore, intensive treatment decreased the albumin excretion rate (AER) by 15% after the first year of therapy (6.5 vs. 7.7  $\mu\text{g}/\text{min}$ ,  $P < 0.001$ ). Thereafter the rates of change for AER within each treatment group were no different from zero, retaining a constant difference in AER between groups in the trial. In the secondary intervention cohort with baseline AER  $< 28 \mu\text{g}/\text{min}$ , intensive therapy reduced the mean adjusted risk of microalbuminuria ( $\geq 28 \mu\text{g}/\text{min}$ ) by 43% (95% CI 21, 58%;  $P < 0.0001$ ); the risk of a more advanced level of microalbuminuria ( $\geq 70 \mu\text{g}/\text{min}$ ) by 56% (95% CI 26, 74%;  $P = 0.002$ ); and the risk of clinical albuminuria ( $\geq 208 \mu\text{g}/\text{min}$ ) by 56% (95% CI 18, 76%;  $P < 0.01$ ). In the secondary intervention cohort, values for AER at year 1 were identical at 9  $\mu\text{g}/\text{min}$ , but the 6.5% change per year in the conventional group greatly exceeded the rate of change of  $-0.3\%$  in the intensive group ( $P < 0.001$ ). Among the 73 secondary cohort subjects with AER levels  $\geq 28 \mu\text{g}/\text{min}$  but  $\leq 139 \mu\text{g}/\text{min}$  at baseline, the reduction of progression to clinical albuminuria with intensive therapy was not statistically significant. The longitudinal treatment effect of conventional versus intensive therapy (11.0% vs. 2.5% per year, respectively,  $P = 0.087$ ) was similar in magnitude to that among patients with AER  $< 28 \mu\text{g}/\text{min}$  at baseline. For the primary, secondary and combined cohorts, there were no significant differences in the rates of change in creatinine clearance ( $C_{Cr}$ ) between treatment groups during the study. Only seven subjects in the entire study (2 intensive, 5 conventional) developed urinary AER  $\geq 208 \mu\text{g}/\text{min}$  coupled with a  $C_{Cr} < 70 \text{ ml}/\text{min}/1.73 \text{ m}^2$ . Neither the rate of change of blood pressure nor the appearance of hypertension (BP  $> 140/90 \text{ mm Hg}$ ) differed significantly between treatment groups in the primary, secondary or combined cohorts. The beneficial effect of intensive therapy on the development of microalbuminuria was consistent in subgroups defined by baseline variables including age, diabetes duration, baseline  $\text{HbA}_{1c}$ , level of retinopathy, neuropathy, and the presence or absence of hyperfiltration ( $C_{Cr} < 130$  or  $\geq 130 \text{ ml}/\text{min}/1.73 \text{ m}^2$ ). Even after adjustment for pregnancy, female subjects nearly demonstrated ( $P = 0.06$ ) a smaller treatment effect than male subjects. In summary, intensive therapy reduced the cumulative incidence and overall risk for the development of microalbuminuria and clinical albuminuria in both the primary prevention and secondary intervention DCCT cohorts. The consistent beneficial effect of

intensive therapy may prevent the onset and will at least delay progression of nephropathy; it may also delay or prevent the development of advanced renal disease in IDDM patients.

Diabetic nephropathy accounts for 25 to 30% of the patients with end-stage renal disease who require dialysis [1–3] and it has been estimated that 30 to 40% of patients with insulin dependent diabetes mellitus (IDDM) will eventually develop end stage renal disease [4–6]. A number of factors may interplay in the pathogenesis of diabetic nephropathy, including metabolic, hemodynamic and, as yet poorly defined genetic and/or environmental determinants.

The Diabetes Control and Complications Trial (DCCT) was a 29 center, randomized clinical trial that compared the effects of intensive diabetes treatment, designed to achieve glucose levels as close to normal as possible, with conventional diabetes treatment on the development and progression of the long-term complications of IDDM [7]. The DCCT studied two cohorts of IDDM patients to answer two separate but related questions: (1.) Would intensive treatment prevent or delay the development of complications in subjects who had no complications at baseline (primary prevention trial); and (2.) Would intensive treatment prevent or slow progression of complications in subjects who had early complications at baseline (secondary intervention trial)? We have previously reported that intensive diabetes therapy delays the onset and slows the progression of retinopathy and delays the development of microalbuminuria ( $\geq 28 \mu\text{g}/\text{min}$ ) in both cohorts and the development of overt nephropathy (albuminuria  $> 208 \mu\text{g}/\text{min}$ ) in the secondary intervention cohort [8]. We now present detailed analyses of changes in renal function in the primary prevention and secondary intervention cohorts, comparing the results in the subjects receiving intensive therapy with those receiving conventional diabetes treatment. These analyses include: [1] life-table analyses which summarize the cumulative incidences of events over time; [2] comparison of treatment groups at specific points in time (point prevalence); and [3] longitudinal analyses of the rates of changes (slopes) of renal function in individuals over time.

## Methods

### Patients

Detailed descriptions of the eligibility criteria and randomization procedures for subjects entering the DCCT as well as their

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baseline renal function have been published [7–9]. All subjects were 13 to 39 years of age and had a duration of IDDM of 1 to 15 years at randomization. They were free of advanced micro- or macrovascular complications of diabetes, had normal glomerular filtration rates evidenced by a serum creatinine <1.2 mg/dl and/or creatinine clearance ( $C_{Cr}$ ) >100 ml/min/1.73 m<sup>2</sup> and were normotensive (BP < 140/90 mm Hg). They had no other significant medical or psychiatric disorders that might complicate their care or limit their participation in the study, were free of neuropathy of sufficient magnitude to require symptomatic treatment, and had calculated LDL cholesterol levels <190 mg/dl. LDL cholesterol was calculated with the Friedewald equation with very-low-density lipoprotein cholesterol (VLDL) concentration assumed to be 20% of triglyceride concentration [10]. Subjects with a history of heavy alcohol consumption within the five years prior to entry or regular use of analgesics were also excluded.

The primary prevention cohort consisted of 726 subjects who fulfilled the following additional selection characteristics at entry: duration of IDDM one to five years, stimulated serum C-peptide levels <0.5 pmol/ml, no retinopathy visible on stereo fundus photography, and a urinary albumin excretion rate (AER) <28 µg/min. The value of 28 µg/min for AER lies in the middle of the range of values cited as being “predictive” of clinical nephropathy [11–14]. At entry, mean duration of diabetes was 2.6 ± 1.4 years, mean age was 26 ± 7 years, mean  $C_{Cr}$  was 127 ± 29 ml/min/1.73 m<sup>2</sup> and median urinary AER was 8 ± 6 µg/min (Table 1 and Fig. 1) [9].

The secondary intervention cohort consisted of 715 subjects who fulfilled the following additional selection characteristics at entry into the study: duration of IDDM 1 to 15 years, stimulated C-peptide levels <0.2 pmol/liter for subjects with five years or more duration of IDDM, minimal to moderate retinopathy (at least 1 microaneurysm in one eye but <P2 by modified Airlie House criteria [15] visible on stereo fundus photography, and urinary AER levels <139 µg/min. At entry, duration of diabetes was 8.7 ± 3.7 years, mean age 27 ± 7 years, mean  $C_{Cr}$  129 ± 31 ml/min/1.73 m<sup>2</sup> and median urinary AER 9.7 ± 12.5 µg/min (Table 1 and Fig. 1). Seventy-three secondary intervention subjects had urinary AER ≥28 µg/min [9].

Baseline characteristics (Table 1 and Fig. 1) did not differ between the treatment groups within each cohort with respect to the variables listed. Data used for the subgroup analyses were collected at baseline and have been described in detail previously [8, 9].

Due to staggered entry of subjects during 1983 to 1989, not all subjects had the same number of measurements by study end. The 278 subjects who entered during the feasibility segment of the trial in 1983 to 1984 provided the longest period of observation of up to nine years. The majority of the additional 1163 patients recruited for the full scale trial were followed for at least four years. The principal analyses employed all 1441 patients entered into the trial, feasibility and full scale phases combined. Additional analyses were performed using only those 278 patients entered during the feasibility phase, 268 of whom were followed for nine years.

#### Assessment of renal function and blood pressure

Details regarding blood pressure measurement, the methods of urine collection and blood collection and measurement of creatinine and albumin and quality control procedures for these

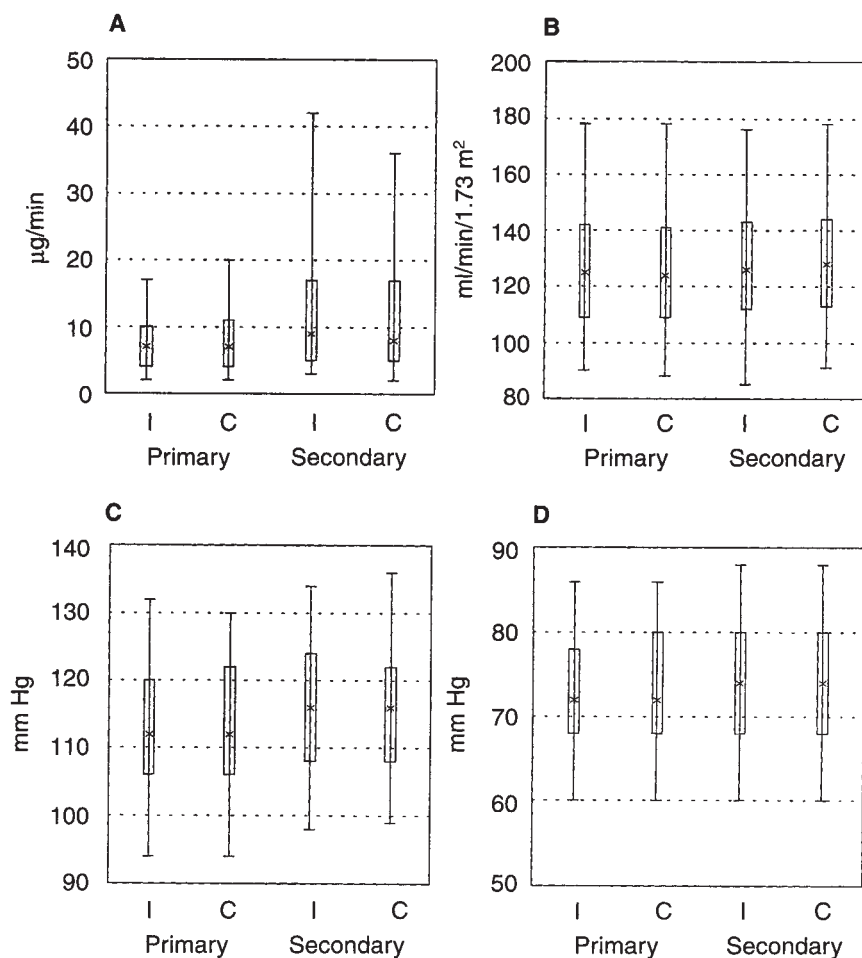
**Table 1.** Selected baseline characteristics

	Primary prevention		Secondary intervention	
	Conventional	Intensive	Conventional	Intensive
<i>N</i>	378	348	352	363
<b>Demographics</b>				
Age years	26 ± 8	27 ± 7	27 ± 7	27 ± 7
Gender (% female)	46	51	46	47
Race (% Caucasian)	96	96	97	97
<b>IDDM</b>				
Duration years	2.6 ± 1.4	2.6 ± 1.4	8.6 ± 3.7	8.9 ± 3.8
HbA1c %	8.8 ± 1.7	8.8 ± 1.6	8.9 ± 1.5	9.0 ± 1.5
<b>Clinical and biochemical features</b>				
Body mass index				
kg/m <sup>2</sup>				
Male	24 ± 3	23 ± 3	24 ± 3	23 ± 3
Female	23 ± 3	23 ± 3	24 ± 3	24 ± 3
Cholesterol mg/dl, serum	173 ± 35	176 ± 33	179 ± 32	178 ± 33
Triglycerides mg/dl, serum	77 ± 57	75 ± 41	87 ± 44	86 ± 45
HDL-cholesterol mg/dl, serum	51 ± 13	52 ± 13	49 ± 11	49 ± 12
LDL-cholesterol mg/dl, serum	106 ± 30	109 ± 29	112 ± 28	112 ± 29
Smoking history %	36	36	36	35
History of kidney infection %	18	18	17	21
Dietary protein g/day/kg	1.7 ± 0.6	1.6 ± 0.5	1.5 ± 0.5	1.6 ± 0.6
<b>Familial</b>				
Family history of diabetes	15	14	14	14
Family history of hypertension %	55	59	57	54
Family history of kidney disease	11	11	8	8
Family history of diabetic kidney disease	3	2	2	2

Data are means ± SD unless otherwise indicated.

methods have been described in detail previously [9]. Briefly, urine collection began after the subjects had breakfast and their morning dose of insulin. If a subject had a hypoglycemic reaction the collection was delayed so that the subject was without symptoms for at least one hour. Subjects were asked to avoid strenuous exercise the day previous to the test. Urine collections were obtained with the subjects resting and in a sitting position and abstaining from caffeinated beverages. Subjects were asked to drink 250 ml of water every half hour during the four hours. The carefully timed specimens were collected on ice, mixed, their total volume measured and aliquots frozen for later assay. Clinic and laboratory quality control were continuously monitored throughout the study by having the laboratory analyze split aliquots from single specimens. For measurements of urine albumin and creatinine and serum creatinine, the respective coefficients of variation were 6.6, 4.0 and 4.0%, and the respective coefficients of reliability were 98, 99 and 86%.

Clinic personnel were masked to the results of renal function measurements. However, if a patient developed a serum creatinine >2.0 mg/dl, the patient and clinic staff were unmasked to this finding so that additional diagnostic tests could be performed or therapeutic measures initiated, if indicated. In the absence of



**Fig. 1.** Baseline distribution of (A) albumin excretion rate (AER), (B) creatinine clearance, and (C) systolic and (D) diastolic blood pressures shown for intensive (I) and conventional (C) treatment subjects in the primary and secondary cohorts. For each parameter the median is shown by an X with the 25th and 75th percentiles shown by the boxes and the 5th and 95th percentiles shown by the bars.

clinical data or renal biopsy findings to the contrary, we assumed that the underlying kidney disease was diabetic nephropathy. There was only one case documented to have nephropathy from a cause other than diabetes during the study.

An increase in urinary AER may be the earliest manifestation or a marker of diabetic nephropathy [11–14]. Therefore, we compared this parameter measured annually between treatment groups in the two cohorts. Urine collections were performed for four hours rather than 24 hours and results for AER will be expressed in terms of  $\mu\text{g}/\text{min}$  rather than  $\text{mg}/24$  hours, as previously reported [8].

Beginning five years after initiation of the DCCT,  $^{125}\text{I}$ -iothalamate clearance studies were performed on all new patients at entry as an additional measurement of GFR. These measurements were performed in most subjects after three years in the study and at study end. The details of the methodology and coefficients of variation have been described previously [16]. All iothalamate and creatinine clearances were adjusted for body surface area at baseline to avoid introducing changes in renal function owing to differences in weight gain that occurred during the trial between the intensive and conventional treatment groups [17].

#### Statistical analysis

All outcomes were analyzed on the basis of original treatment assignment. Major events of interest were defined prior to study

end while the study group was still masked to study results. These events were the following: albuminuria  $>28$   $\mu\text{g}/\text{min}$  (40  $\text{mg}/24$  hr),  $>70$   $\mu\text{g}/\text{min}$  (100  $\text{mg}/24$  hr), and  $>208$   $\mu\text{g}/\text{min}$  (300  $\text{mg}/24$  hr). The development of renal insufficiency was defined as the observation of an AER  $>208$   $\mu\text{g}/\text{min}$  and a  $C_{\text{Cr}} <70$   $\text{ml}/\text{min}/1.73$   $\text{m}^2$  on the annual evaluation. Hypertension was defined as a sitting systolic blood pressure  $>140$  mm Hg or diastolic blood pressure  $>90$  mm Hg on two consecutive readings one month apart. Event rates are presented as number per 100 patient years based on the ratio of the observed number of patients experiencing the event (cases) to the total patient years of exposure (at risk). The lifetable method was used to estimate the cumulative incidence of events [18] with adjustments for periodically timed assessments [19].

The average relative risk comparing the two treatment groups within each of the primary and secondary cohorts over the complete period of observation was estimated by a proportional hazards (PH) regression model [18], with adjustment for the numerical baseline value. The Wald test from the PH model was used to test the difference between cumulative incidence curves. The adjusted percentage reduction in risk for intensive therapy versus conventional therapy was calculated from the average adjusted relative risk (RR) of intensive versus conventional treatment as  $(1 - \text{RR}) \times 100\%$ . The relative risk in the combined cohorts was estimated with stratification by primary and secondary cohorts.

The Wilcoxon rank sum test was used to compare the treatment groups with respect to the distributions of ordinal or numerical variables, and the contingency  $\chi^2$  test for categorical variables [20]. For the analysis of repeated measurements over time of an ordinal or a numerical variable, the multivariate Wilcoxon analysis [21] was employed in conjunction with an overall test of differences between groups using weights proportional to the total sample size at each annual evaluation [22]. The repeated measures analysis of AER and  $C_{Cr}$ , employed a covariance adjustment for the baseline values on the log scale. Because iothalamate clearance was not measured at baseline in the majority of patients, analyses of iothalamate clearance did not employ a covariance adjustment for baseline values.

The distributions of AER at baseline were strongly positively skewed, demonstrated by means that were somewhat larger than their respective medians. Thus, the natural log (ln) transformation was employed to improve the distributions of these measures. However, the mean, standard deviation (SD) and percentiles of the ln(measures), are not as readily interpretable as those in the original units of measurement. Thus, the exponential function of the mean on the ln scale was used to convert the mean of the ln(measures) back to the original units of measurement. This yields the geometric mean which is an estimate of the median under a ln-normal distribution. In general, these geometric means corresponded favorably to the medians of the original nontransformed data (Table 4).

Random effects models for the analysis of longitudinal data (so-called growth curve models) were employed to describe the rates of change of renal function measures (albuminuria, creatinine clearance and blood pressure) over time [23, 24]. The basic model for each patient was of the form:  $\log(y_t) = \alpha + \beta(t - 1)$ , where  $t$  represents the year of evaluation. Thus the intercept ( $\alpha$ ) reflects the fitted value at year 1. Since the natural log of  $y$  was employed, the slope  $\beta * 100$  is approximately the relative (%) change per year. These analyses employed the baseline level of the measure as a covariate in the model because eligibility restrictions on these measures would lead to biased estimates if the baseline values were included in the slope calculations [25].

In these models, the treatment effect was partitioned into two components: the acute effect of treatment and the long-term effect. The acute effect of intensive versus conventional treatment is reflected by a difference between groups at the first year of follow-up. Time ( $t$ ) is re-scaled as  $t - 1$  so that  $t - 1 = 0$  corresponds to year 1 of follow-up. Then the average intercept for the subjects in a treatment group gives an estimate of the geometric mean at year 1 of follow-up. The difference between these average group intercepts, or the ratio of the geometric means, provides an estimate of the acute treatment effect. For each subject a regression model is fit to all values from year 1 and later, with subjects having differing numbers of follow-up measures based on their length of participation in the trial. Then the difference between groups in the average slopes (relative % change per year) reflects the differences in trends beyond the first years of follow-up.

The Appendix describes in greater detail the models employed and the precise estimates of the model coefficients and variance components for the analyses of AER. This detailed information is provided to assist others who may wish to conduct similar analyses of data from other trials, or more importantly, to use the parameter estimates of the coefficients and variance components

for the planning of future clinical trials of pharmacologic or therapeutic interventions in the treatment of diabetic nephropathy. The analyses were performed using the program 5V of the BMDP [26].

For the analyses within a cohort (that is, the primary or the secondary cohort), the model included the baseline value as a covariate, treatment group as a group effect, and the intercept and time (slope) as random effects, and a group by time interaction. For subgroup analyses (such as by gender), separate analyses were performed within each subgroup (for example, for males and for females). An additional model was employed with the subgrouping covariate (such as gender) and its interactions with the above factors added to the model so as to provide a test of the covariate by treatment interaction (homogeneity).

Results are presented as mean  $\pm$  SD unless otherwise noted. All results nominally significant at  $P < 0.05$  are indicated.

## Results

There were no significant differences in baseline data between the treatment groups in either the primary prevention or the secondary intervention cohorts (Table 1). The distributions of AER,  $C_{Cr}$ , and systolic and diastolic blood pressures at baseline are given in Figure 1. Baseline iothalamate clearance performed in 476 subjects was 128 ( $\pm 20$ ) ml/min/1.73 m<sup>2</sup> and did not differ among the randomized treatment groups.

### *Extent of follow-up and adherence to assigned treatment*

The mean duration of follow-up for the entire cohort of 1441 subjects was six and one-half years with a range of three to nine years. Two patients did not undergo any follow-up renal assessments: one intensively treated patient in the primary cohort, and one conventionally-treated patient in the secondary cohort. More than 2000 patient years were accrued in each treatment group for each cohort for a total of approximately 9300 patient years of observation. In the various life-table analyses, however, the total patient-years "at risk" are somewhat less than the total years of follow-up because exposure time is calculated up to the time of the event (or end of the study). Follow-up in the study was virtually complete. End of study data were collected on 1422 subjects, 99% of the total cohort. The reasons for subjects failing to complete the study included 11 who died and 8 drop-outs.

The subjects' adherence to their randomly assigned treatment was also high. Overall, subjects randomly assigned to intensive treatment spent more than 98% of their time using intensive therapy and subjects assigned to conventional treatment spent more than 97% of time during the study using conventional therapy. This included 95 women who deviated from their assigned conventional treatment according to protocol in preparation for and during pregnancy. Conventional therapy was resumed when the pregnancy ended in all women assigned to conventional therapy.

At baseline, mean HbA<sub>1c</sub> levels were similar in both treatment groups (Table 1). By three months after randomization, mean HbA<sub>1c</sub> was approximately 2% lower in the intensive treatment group than the conventional treatment group and this difference was maintained throughout the study, (7.2 vs. 9.1%,  $P < 0.001$ ).

### *Urinary albumin excretion*

*Cumulative incidence analyses.* We compared the intensively and conventionally treated subjects for the development of various levels of AER. Table 2 shows the incidence rates and the risk

**Table 2.** Summary of absolute rates for development of levels of AER and percentage risk reduction with intensive therapy

	Primary prevention % <sup>a</sup>				Secondary intervention % <sup>a</sup>				Combined cohorts <sup>b</sup>	
	Conv	Int	Reduction	(95% CI)	Conv	Int	Reduction	(95% CI)	Reduction	(95% CI)
<b>A. Patients with AER &lt; 28 µg/min at baseline<sup>c</sup></b>										
Albuminuria ≥ 28 µg/min (40 mg/24 hrs)										
# Cases	67	41			99	69				
Rate/100 py	3.4	2.2	34	(2, 56) <sup>a</sup>	5.7	3.6	43	(21, 58) <sup>h</sup>	39	(21, 52) <sup>h</sup>
Cum % @ 9y	27.3	16.0			42.1	26.2				
Sustained <sup>c</sup> albuminuria ≥ 28 µg/min										
# Cases	18	7			49	23				
Rate/100 py	0.9	0.4	56	(-5, 82)	2.53	1.1	61	(36, 77) <sup>h</sup>	60	(37, 74) <sup>h</sup>
Cum % @ 9y	6.9	2.7			18.6	7.4				
Albuminuria ≥ 70 µg/min (100 mg/24 hrs)										
# Cases	18	10			43	22				
Rate/100 py	0.9	0.5	39	(-34, 72)	2.2	1.03	56	(26, 74) <sup>h</sup>	51	(24, 68) <sup>h</sup>
Cum % @ 9y	7.0	3.3			20.2	10.0				
Sustained <sup>c</sup> albuminuria ≥ 70 µg/min										
# Cases	5	2			23	9				
Rate/100 py	0.2	0.10	54	(-136, 91)	1.1	0.4	67	(28, 84) <sup>h</sup>	65	(29, 83) <sup>h</sup>
Cum % @ 9y	7.0	3.3			8.3	3.1				
<b>B. All patients<sup>d</sup></b>										
Albuminuria ≥ 208 µg/min (300 mg/24 hrs)										
# Cases	6	3			31	15				
Rate/100 py	0.3	0.2	44	(-124, 86)	1.4	0.6	56	(18, 76) <sup>h</sup>	54	(19, 74) <sup>h</sup>
Cum % @ 9y	2.3	2.6			11.3	5.2				
Sustained <sup>c</sup> albuminuria ≥ 208 µg/min										
# Cases					22	11				
Rate/100 py <sup>f</sup>					1.0	0.5	56	(8, 79) <sup>h</sup>	51	(2, 75) <sup>h</sup>
Cum % @ 9y					7.2	3.2				

<sup>a</sup> Percent reduction: percentage risk reduction of intensive compared with conventional treatment calculated from a proportional hazards model adjusted for baseline AER values

<sup>b</sup> Stratified by primary prevention vs. secondary intervention

<sup>c</sup> Patients with AER > 28 µg/min at baseline excluded, including one intensive-treated patient in the primary cohort.

Primary Prevention Cohort (Conventional  $N = 378$ ; pt years = 2192; Intensive  $N = 346$ ; pt years = 2010)

Secondary Intervention Cohort (Conventional  $N = 316$ ; pt years = 2367; Intensive  $N = 325$ ; pt years = 2468)

Total patient years of follow-up of AER assessments (0–9 yr per patient). Patient years at risk slightly less in each analysis depending on the number and timing of events.

<sup>d</sup> Two patients excluded who did not complete at least one annual evaluation.

Primary Prevention Cohort (Conventional  $N = 378$ ; pt years = 2192; Intensive  $N = 347$ ; pt years = 2010)

Secondary Intervention Cohort (Conventional  $N = 351$ ; pt years = 2367; Intensive  $N = 363$ ; pt years = 2468)

<sup>e</sup> Event observed at two successive annual evaluations.

<sup>f</sup> Number of cases too small (< 5) to allow meaningful analysis.

<sup>g</sup>  $P < 0.05$ , two-tailed, <sup>h</sup>  $P < 0.01$ , two tailed

reduction with intensive therapy. Table 3 shows the incidence rates partitioned over time. Figures 2 to 4 show the cumulative incidence curves. In the following sections the results of these analyses are described.

#### Microalbuminuria ≥ 28 µg/min

**Primary prevention cohort.** Of the 346 intensively treated subjects, 41 developed microalbuminuria during 1876 person-years at risk, for an overall hazard rate of 2.2 per 100 patient-years at risk. By lifetable analysis, the cumulative incidence of microalbuminuria after nine years was estimated to be 16% (Table 2). Of the 378 conventional therapy subjects, 67 developed microalbuminuria during 1987 person-years at risk, for an overall hazard rate of 3.4 per 100 patient-years at risk and a nine-year estimated cumulative incidence of 27%. The difference in the cumulative incidence curves (Fig. 2A) between the two treatment groups was significant ( $P = 0.04$ ). The average relative risk (conventional vs. intensive), adjusted for baseline AER levels, was 1.51 with a 95%

confidence interval (CI) of 1.02, 2.25. This corresponds to a 34% reduction in risk with intensive treatment (95% CI: 2,56). Whereas the rate of cases developing microalbuminuria in the conventionally treated group increased modestly over time, the converse was seen in the intensively treated group, resulting in a rather striking increase in the relative risk (Conventional vs Intensive) over time (Table 3).

**Secondary intervention cohort.** Sixty-nine of the 325 intensively treated subject with baseline AER < 28 µg/min developed microalbuminuria during 1940 person-years at risk, for an overall hazard rate of 3.6 per 100 patient-years at risk. The cumulative incidence after nine years was 26%. Of the 316 conventional therapy subjects with baseline AER < 28 µg/min, 99 developed microalbuminuria during 1736 person-years at risk, for an overall hazard rate of 5.7 per 100 patient-years at risk. The nine-year cumulative incidence was 42%. As in the primary prevention cohort, the difference in the cumulative incidence curves (Fig. 2b) between the two treatment groups was significant ( $P < 0.001$ ).

Table 3. Incidence of nephropathy events for different time periods

Event	Years of study time	Conventional (C)				Intensive (I)				Relative risk: C vs. I	
		N	Cases	Per yrs	Rate	N	Cases	Per yrs	Rate	Unadjusted	Adjusted <sup>a</sup>
AER > 28 µg/min in primary cohort with baseline AER < 28 µg/min	0-1	378	10	378	2.65	346	11	346	3.18	0.83	
	2-3	367	26	719	3.62	335	18	659	2.73	1.32	
	4-5	339	17	597	2.85	314	7	548	1.28	2.23	
	6-9	143	14	293	4.78	148	5	323	1.55	3.09	
	0-9	378	67	1987	3.37	346	41	1876	2.19	1.56 (1.05, 2.32) <sup>b</sup>	1.51 (1.02, 2.25)
AER > 28 µg/min in secondary cohort with baseline AER < 28 µg/min	0-1	316	19	316	6.01	325	18	325	5.54	1.09	
	2-3	297	39	572	6.82	307	24	597	4.02	1.70	
	4-5	258	26	500	5.20	281	14	555	2.52	2.06	
	6-9	167	15	348	4.31	204	13	463	2.81	1.54	
	0-9	316	99	1736	5.70	325	69	1940	3.56	1.62 (1.18, 2.22)	1.74 (1.26, 2.39)
AER > 208 µg/min in all patients	0-1	729	2	729	0.27	710	4	710	0.58	0.49	
	2-3	726	8	1448	0.55	705	5	1404	0.36	1.55	
	4-5	714	14	1336	1.05	694	4	1301	0.31	3.41	
	6-9	417	13	938	1.39	437	5	1002	0.50	2.78	
	0-9	729	37	4451	0.83	710	18	4417	0.41	2.07 (1.17, 3.63)	2.18 (1.23, 3.85)

Rate is cases per 100 person-years.

<sup>a</sup> Within Primary and Secondary Cohorts, adjusted for log of AER at baseline as a continuous variable. In the analysis of all patients also adjusted for primary and secondary cohort status.

<sup>b</sup> Relative risk (95% confidence interval)

The adjusted relative risk (conventional vs. intensive) was 1.74 (95% CI: 1.26, 2.39), giving a mean reduction in absolute risk with intensive therapy of 43% (95% CI 21, 58%). In the secondary cohort, the increase in the relative risk with time was less pronounced than in the primary intervention cohort (Table 3).

**Combined cohorts.** Of the 671 intensively treated subjects with baseline AER <28 µg/min, 110 developed microalbuminuria. For the 694 conventionally treated subjects with baseline AER <28 µg/min, 166 developed microalbuminuria. The difference in the cumulative incidence curves between the two treatment groups in the total study was significant ( $P < 0.001$ ). The adjusted relative risk (conventional vs. intensive) was 1.63 (95% CI: 1.27, 2.08), giving a reduction in absolute risk with intensive therapy of 39% (95% CI: 21, 52) (Table 2).

#### Microalbuminuria $\geq 70$ µg/min

We also explored the risk of developing more advanced levels of microalbuminuria among those who had baseline AER <28 µg/min at baseline.

**Primary prevention cohort.** Ten intensively treated subjects developed an AER >70 µg/min (Fig. 3A). This resulted in a cumulative incidence after nine years of 3.3% and an overall hazard rate of 0.5 per 100 patient-years at risk (Table 2). Eighteen conventional therapy subjects developed AER >70 µg/min (Fig. 3A). The cumulative incidence and overall hazard rate were 7.0% and 0.9 per 100 patient years, respectively.

**Secondary intervention cohort.** Twenty-two intensively treated subjects with baseline AER <28 µg/min developed an AER >70 µg/min (Fig. 3B). This resulted in a cumulative incidence of 10.0% after nine years and an overall hazard rate of 1.03 per 100 patient-years at risk. Forty-three conventional therapy subjects developed an AER >70 µg/min (Fig. 3B). The cumulative incidence and overall hazard rate were 20.2% and 2.2 per 100 patient years respectively. The difference in the cumulative incidence curves between the two treatment groups was significant at

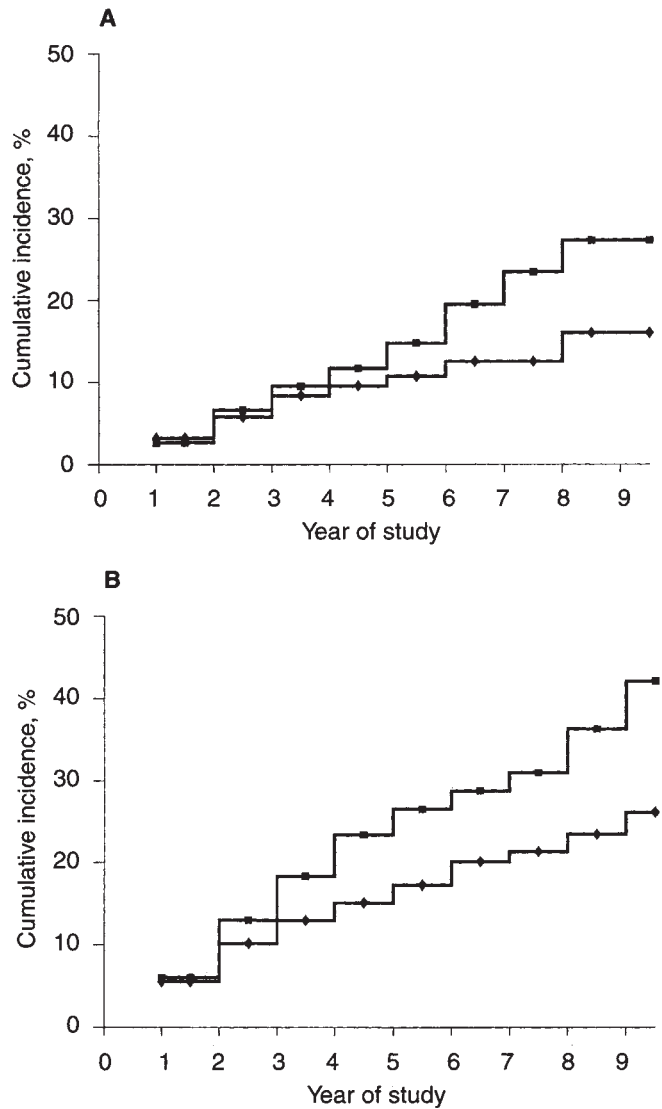
$P = 0.002$  (Fig. 3B). The adjusted relative risk (conventional vs. intensive) was 2.28 (95% CI: 1.35, 3.85), giving a mean reduction in absolute risk with intensive therapy of 56% (95% CI: 26, 74%) (Table 2).

**Combined cohorts.** Of the 671 intensively treated subjects with baseline AER <28 µg/min, 32 developed an AER >70 µg/min. Sixty-one of the 694 conventional treatment subjects with baseline AER <28 µg/min developed an AER >70 µg/min. The adjusted relative risk (conventional vs. intensive) was 2.03 (95% CI: 1.32, 3.14), giving a mean reduction in absolute risk with intensive therapy of 51% (95% CI: 24, 68,  $P < 0.001$ ; Table 2).

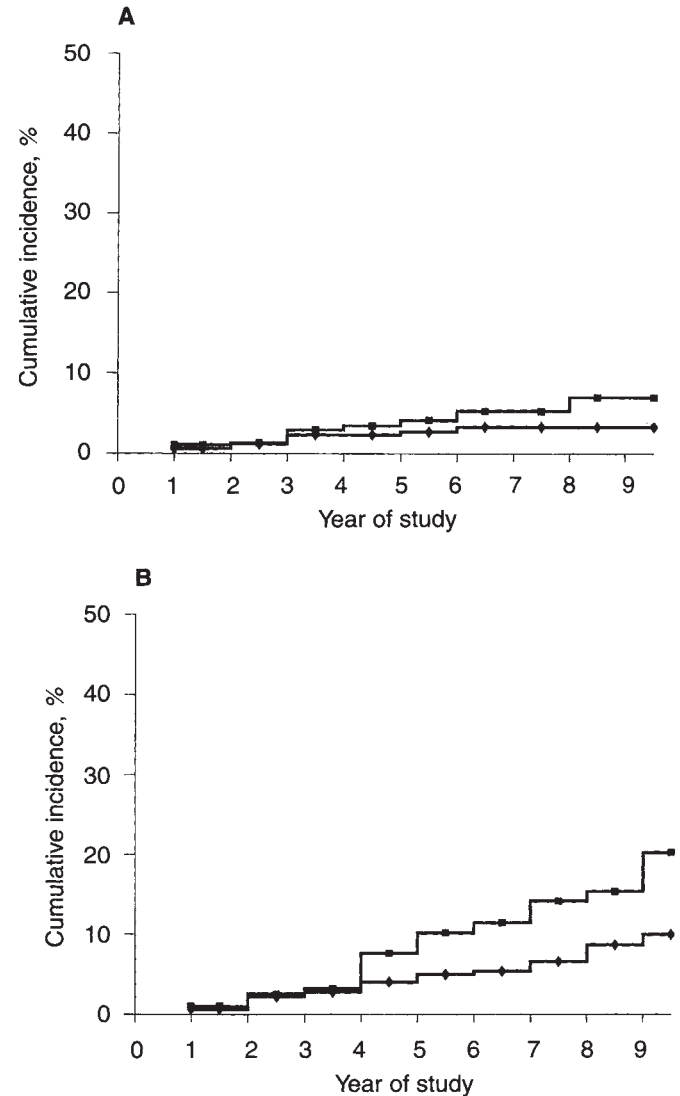
#### Clinical albuminuria $\geq 208$ µg/min

**Primary prevention cohort.** Only three intensively treated subjects developed clinical albuminuria (cumulative incidence after 9 years of 2.6% and an overall hazard rate of 0.15 per 100 patient-years at risk; Table 2). Six conventional therapy subjects developed clinical albuminuria. The cumulative incidence and overall hazard rate were 2.3% and 0.28 per 100 patient years, respectively. The cumulative incidence curves were not significantly different.

**Secondary intervention cohort.** Fifteen of 363 intensively treated subjects developed clinical albuminuria. This resulted in a cumulative incidence after nine years of 5.2% and an overall hazard rate of 0.62 per 100 patient-years at risk (Table 2). Of 357 conventional treatment subjects, 31 developed clinical albuminuria. The cumulative incidence and overall hazard rate were 11.3% and 1.36 per 100 patient years, respectively. The difference in the cumulative incidence curves between the two treatment groups was significant ( $P < 0.01$ ; Fig. 4). The adjusted relative risk (conventional vs. intensive) was 2.27 (95% CI: 1.21, 4.23), giving a mean reduction in absolute risk with intensive therapy of 56% (95% CI: 18, 76%; Table 2). As with microalbuminuria, this relative risk was not constant over time in the study (Table 3). Although numbers in each year were relatively small, there was a



**Fig. 2.** Cumulative incidence of development of microalbuminuria (AER  $>28 \mu\text{g}/\text{min}$ ) in the intensive (diamonds) and conventional (■) treatment groups. **A.** Primary prevention cohort ( $P = 0.04$ ). **B.** Secondary intervention cohort (among those with baseline AER  $<28 \mu\text{g}/\text{min}$ ;  $P = 0.001$ ).



**Fig. 3.** Cumulative incidence of development of AER  $>70 \mu\text{g}/\text{min}$  in the intensive (diamonds) and conventional (■) treatment groups. **A.** Primary prevention cohort ( $P = 0.219$ ). **B.** Secondary intervention cohort (among those with baseline AER  $<28 \mu\text{g}/\text{min}$ ;  $P = 0.002$ ).

tendency for a decrease in the rate of development of clinical albuminuria in the intensively treated group in the secondary intervention cohort, contrasting to an increase in the rate in the conventionally treated group. This resulted in a trend toward an increase in the relative risk over time.

#### Sustained microalbuminuria and clinical albuminuria

Subjects reaching the stipulated levels of AER (28, 70 and  $208 \mu\text{g}/\text{min}$ ) on two successive annual evaluations were designated to have *sustained* elevations of AER. The hazard rates, cumulative percentages at nine years and risk reductions for these sustained elevations are given in Table 2. Intensive therapy reduced the average risk for development of sustained outcomes by 51 to 67%, levels slightly greater than the less stringent single measurement outcomes.

#### Point prevalence analyses

The variability of AER is well known and some subjects recover from microalbuminuria or even clinical albuminuria. Unlike the analysis of cumulative incidence, the analysis of prevalence reflects the status of a patient at a given visit regardless of the status at other visits and incorporates the possibility of recovery. Figure 5 A and B show the point prevalence or the percent of subjects in each of the categories, 28 to  $70 \mu\text{g}/\text{min}$ , 70 to  $208 \mu\text{g}/\text{min}$ , and  $>208 \mu\text{g}/\text{min}$ , at each annual follow-up visit in the primary prevention and secondary intervention cohorts. In both cohorts, the relatively steep increase in percent of conventional treatment group patients with microalbuminuria or clinical albuminuria contrasts with the more gradual increase in the intensive treatment group. Formal tests of the overall difference between the

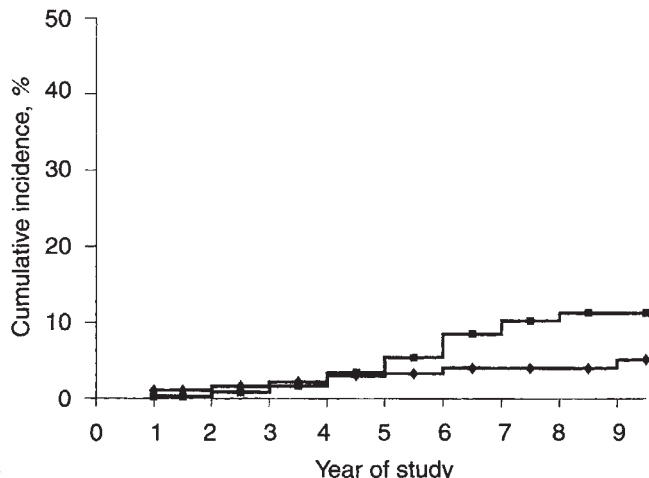


Fig. 4. Cumulative incidence of development of clinical albuminuria AER >208 µg/min among all subjects in the intensive (diamonds) and conventional (■) treatment groups in the secondary intervention cohort (P = 0.01).

two treatment groups over time were significant (P < 0.001 in each of the primary prevention and secondary intervention cohorts).

*Analyses of rates of change of AER*

**Primary prevention cohort.** Within the intensively-treated group, the geometric mean (ln-normal median) was stable over time (Table 4). However, the upper quartile increased over time, indicating increasing spread (and positive skewness) in the distribution of the measures. Within the conventionally treated group, both the mean and upper quartile increased with time.

The analysis of longitudinal changes in AER was divided into the acute effects of intensive therapy (at one year of therapy) and the long-term effects (after the first year). Using a growth curve analysis in the primary prevention cohort on the ln scale (see **Methods** and detailed model described in the **Appendix**), the difference in intercepts between treatment groups provides a direct estimate of the ratio of the geometric means at year 1 (Table 5). The estimated ratio of 0.85 (significantly different from 1, P < 0.0001) indicated that the estimated geometric mean (median) of AER after one year of treatment is 15% less among intensively treated than conventionally treated subjects. The fitted model also provides an estimate of the individual group geometric means (medians) at year 1, and the group difference which is estimated to be -1.14 µg/min.

The long-term trend is reflected by the average slope of the ln(AER) per year of follow-up beyond the first year. This is equivalent to the average relative (or percent) change in AER per year, estimated to be -1.31%/year in the intensive treatment group and -0.52%/year in the conventional treatment group (Table 5). The long-term effect of intensive versus conventional treatment is the difference in mean slopes which is -0.79%/year (P = 0.51). Thus, the AER in both treatment groups showed a slight trend to decrease with time, but neither trend was significantly different from zero (no change over time), or each other.

The overall difference between intensive versus conventional treatment in the primary cohort, therefore, is reflected by the acute effect of treatment at year 1 rather than a long-term effect

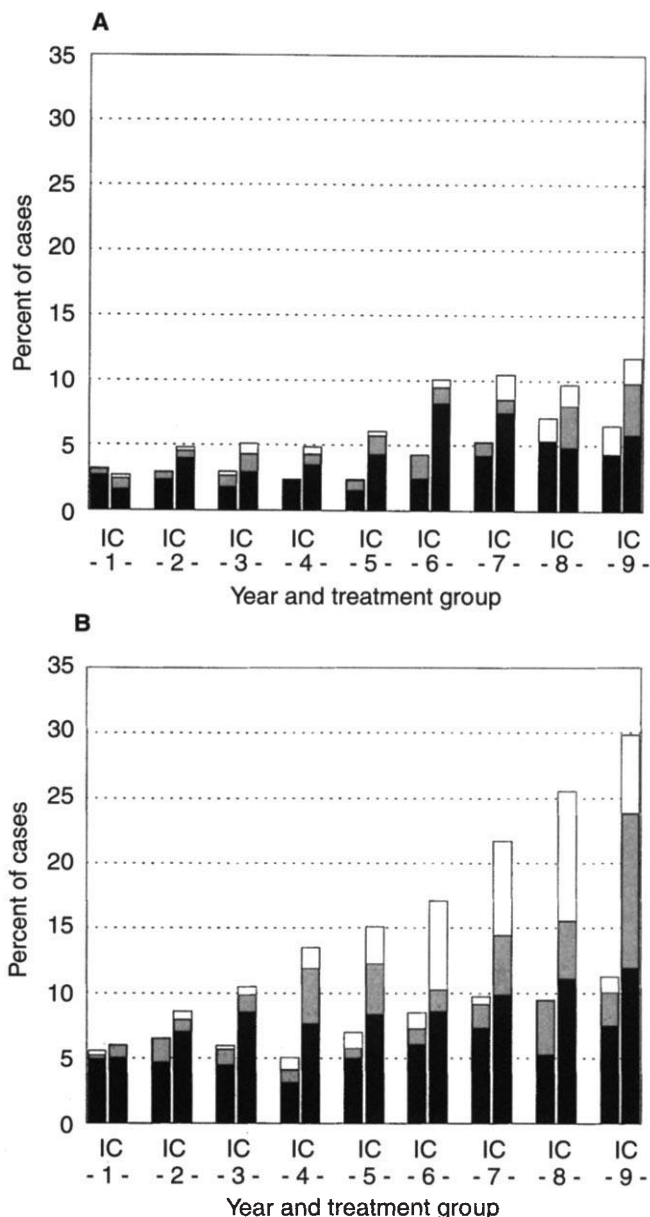


Fig. 5. Point prevalence of albuminuria at different levels [(□) 28–70, (vertical striped box) 70–208, and (■) 208 µg/min] over the course of the study in the intensive (I) and conventional (C) treatment groups. A. Primary prevention cohort. B. Secondary intervention cohort. Overall differences between treatment groups were significant for primary and secondary cohorts (P < 0.001).

on the slopes. A bivariate test of both temporal components of this overall difference between intensive and conventional therapy is highly significant (P < 0.0001; Table 5), principally due to the acute effects of intensive treatment. An alternate explanation would be that only a subset of patients benefit by intensive therapy and the difference between treatment groups is diluted out when the rate of AER in all patients is considered.

Even though the distributions of slopes in the two treatment groups did not differ significantly (Fig. 6A), a large fraction of the



**Table 4.** Urinary albumin excretion rate ( $\mu\text{g}/\text{min}$ ) over time presented as geometric mean and estimated quartiles from a ln-normal distribution

Time in years	N	Intensive			Conventional			
		Geometric mean	25%	75%	N	Geometric mean	25%	75%
<b>A. Primary cohort</b>								
0	348	6.6	4.3	10.2	378	6.7	4.2	10.5
1	347	6.5	4.2	10.1	374	7.4	4.5	12.2
2	344	6.5	4.2	10.2	374	8.0	5.0	12.9
3	340	6.4	3.9	10.4	372	7.5	4.3	13.1
4	343	6.0	3.8	9.4	371	7.6	4.4	13.1
5	254	6.4	4.0	10.2	280	7.4	4.4	12.6
6	165	6.7	3.9	11.5	169	8.4	4.3	16.3
7	96	6.9	3.9	12.3	105	8.7	4.6	16.5
8	57	7.3	3.6	14.7	62	9.3	4.5	19.0
9	47	6.6	3.0	14.7	51	9.9	4.3	22.8
<b>B. Secondary cohort</b>								
0	363	9.5	5.3	17.2	352	9.1	5.1	16.2
1	361	8.9	5.0	16.1	351	9.0	5.0	16.1
2	358	9.0	4.8	16.9	348	9.7	5.2	17.8
3	354	8.9	4.7	16.8	349	10.5	5.3	20.9
4	352	9.4	4.8	18.1	346	11.7	5.4	25.4
5	351	9.0	4.4	18.6	345	12.0	5.2	27.6
6	276	9.4	4.6	19.4	262	13.8	5.4	35.4
7	186	9.5	4.6	19.5	171	16.4	6.2	43.9
8	106	9.3	4.2	20.6	103	18.4	6.3	53.5
9	91	9.5	4.2	21.9	79	18.3	6.2	54.2

patients in each treatment group showed positive rates of progression: 43% in the intensive and 45% in the conventional groups. Approximately 10% of conventional subjects and 8% of intensive subjects showed at least a 20% increase per year (a fourfold increase over 8 years). However, the acute effect of intensive treatment was to reduce the AER values at year 1 by 15% relative to those with conventional treatment. Thus, intensively treated patients who developed increased AER (positive slopes), took longer to reach defined levels of AER (such as a value  $>208 \mu\text{g}/\text{min}$ ) than did patients in the conventional group who had positive slopes because their AER values were 15% lower at year 1. In the aggregate, all of these differences translated into intensive treatment reducing the risk of reaching microalbuminuria and clinical albuminuria by life-table analysis.

**Secondary intervention cohort.** In contrast to the primary prevention cohort, there was no difference in the acute treatment effect between treatment groups at year 1; the ratio of geometric means equaled 0.96 (Table 5). Thereafter values in the conventionally-treated group rose on average 6.46% per year (a 65% increase over 8 years), significantly greater than the average rate of change among intensive subjects ( $-0.25\%$  per year) for a difference between treatment groups of 6.72% per year ( $P < 0.001$ , Table 5). Of the distribution of slopes, 47% were positive in the intensive versus 58% in the conventional group (Fig. 6B). Also, 13% of conventional subjects experienced at least a 30% increase per year (an 8.2-fold increase over 8 years) versus only 3% of intensive subjects. These differences translated into intensive therapy reducing the risk of reaching clinical grade albuminuria by 56% compared with conventional therapy by life-table analysis (Table 2). Similar results were obtained in a separate analysis within the Phase II secondary intervention cohort.

**Table 5.** Summary of regression analysis of  $\ln(\text{AER})$  over time, adjusted for baseline  $\ln(\text{AER})$ 

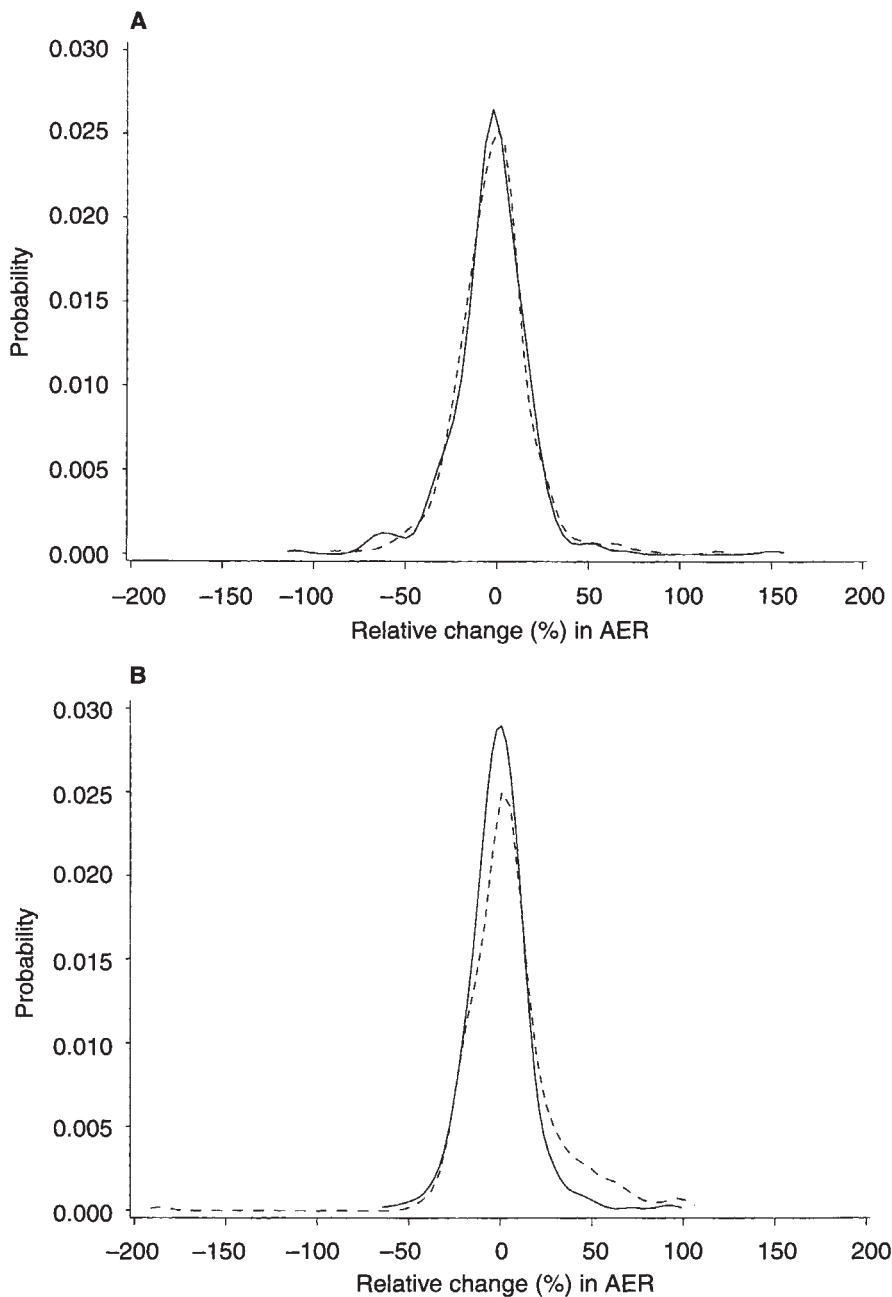
	Estimate	95% C.I.	$P <$
<b>A. Primary cohort</b>			
Year 1 values (Intercept)			
Ratio of geometric means (I:C)	0.85	(0.92, 0.79)	0.001
Fitted geometric means			
Intensive ( $\mu\text{g}/\text{min}$ )	6.54	(6.18, 6.93)	
Conventional ( $\mu\text{g}/\text{min}$ )	7.68	(7.27, 8.11)	
Difference (I-C)	-1.14		
Relative (%) change (slope)			
Intensive	-1.31	(-3.02, 0.40)	0.133
Conventional	-0.52	(-2.16, 1.12)	0.535
Difference (I-C)	-0.79	(-3.16, 1.58)	0.513
Overall test for treatment group effect (intercept and/or slope), 2df:			0.001
<b>B. Secondary cohort</b>			
Year 1 values (intercept)			
Ratio of geometric means (I:C)	0.96	(1.06, 0.88)	0.453
Fitted geometric means			
Intensive ( $\mu\text{g}/\text{min}$ )	9.03	(8.46, 9.65)	
Conventional ( $\mu\text{g}/\text{min}$ )	9.37	(8.76, 10.01)	
Difference (I-C)	-0.33		
Relative (%) change (slope)			
Intensive	-0.25	(-2.27, 1.76)	0.806
Conventional	6.46	(4.40, 8.52)	0.001
Difference (I:C)	-6.72	(-9.60, -3.83)	0.001
Overall test for treatment group effect (intercept and/or slope), 2df:			0.001

**Feasibility cohort.** A separate analysis was performed within the feasibility phase cohort (both primary and secondary) ( $N = 278$ ) in which the majority of patients were followed for nine years. Here the acute effect of intensive treatment was a 16% lower mean AER at one year compared with conventional treatment. Thereafter AER increased at a rate of 3.4%/year in the conventional group compared with 1.1%/year in the intensive group ( $P = 0.35$ ). The 3.4% annual increase in the conventional group is significantly different from zero.

#### Analyses in subjects with AER $>28 \mu\text{g}/\text{min}$ at entry

In the secondary intervention cohort, 73 (38 treated intensively, 35 treated conventionally) subjects had AER levels  $>28 \mu\text{g}/\text{min}$ , but  $<139 \mu\text{g}/\text{min}$ , at the time of entry into the study. In each treatment group, eight developed AER  $>208 \mu\text{g}/\text{min}$  at one or more time points during the course of the study. In the intensively treated group, AER levels had returned to normal by the end of the study in 23 subjects, 11 maintained levels in the microalbuminuric range and 4 subjects developed clinical albuminuria. In the conventionally treated subjects, AER levels had returned to normal in 18, 11 maintained levels in the microalbuminuric range and 6 subjects developed clinical albuminuria. These differences in the cumulative incidence of these events were not statistically significant, nor were differences in the distributions of the AER at any point in time.

In these 73 subjects the difference in mean slope with intensive versus conventional therapy, albeit not significant ( $P = 0.09$ ), was similar in magnitude (8.55%/year) to the difference in mean slope with intensive versus conventional in those 642 secondary cohort subjects (6.5%/yr,  $P < 0.0001$ ) who entered with AER  $<28 \mu\text{g}/\text{min}$ .



**Fig. 6.** Distribution of slopes for urinary albumin excretion, expressed as relative percent change per year. The dashed line represents the distribution of subjects on conventional therapy; the solid line, those on intensive therapy. **A.** Primary prevention cohort. **B.** Secondary intervention cohort.

#### *Creatinine clearance*

For the primary, secondary and combined cohorts, there were no significant differences in creatinine clearance between treatment groups during the study. Slope analyses using log (ln) transformed data similarly did not reveal differences between the treatment groups in either the primary prevention or secondary intervention cohorts (Table 6). The number of events in which subjects experienced urinary AER  $>208 \mu\text{g}/\text{min}$  with a  $C_{Cr} <70 \text{ ml}/\text{min}/1.73 \text{ m}^2$  were as follows: primary prevention cohort – 0 in the intensive and 1 in the conventional groups; secondary intervention cohort – 2 in the intensive and 4 in the conventional groups.

#### *Iothalamate clearance*

Iothalamate clearance was slightly but not significantly lower during follow-up in the intensive treatment groups compared with the conventional treatment groups ( $P = 0.084$  in the primary cohort and  $P = 0.126$  in the secondary cohort) (Table 7). For example, at year 3, the median iothalamate clearances in the intensively and conventionally treated groups were 123 and 125  $\text{ml}/\text{min}/1.73 \text{ m}^2$ , respectively, in the primary cohort and 119 and 123  $\text{ml}/\text{min}/1.73 \text{ m}^2$ , respectively, in the secondary cohort. At study end, the median iothalamate clearances in the primary cohort were 125 and 126  $\text{ml}/\text{min}/1.73 \text{ m}^2$  in the intensively and conventionally treated groups, respectively, and 121 in intensively

**Table 6.** Summary of regression analysis of ln(creatinine clearance) over time, adjusted for baseline ln(creatinine clearance)

	Estimate	95% C.I.	P <
<b>A. Primary cohort</b>			
Year 1 values (intercept)			
Ratio of geometric means (I:C)	1.01	(1.04, 0.99)	
Fitted geometric means			
Intensive (ml/min/1.73 m <sup>2</sup> )	124.82	(122.64, 127.04)	
Conventional (ml/min/1.73 m <sup>2</sup> )	123.45	(121.37, 125.57)	
Difference (I-C) (ml/min/1.73 m <sup>2</sup> )	1.37		
Relative (%) change (slope)			
Intensive	0.27	(-0.21, 0.75)	0.272
Conventional	0.39	(-0.08, 0.85)	0.104
Difference (I-C)	-0.12	(-0.78, 0.55)	0.733
Overall test for treatment group effect (intercept and/or slope), 2df:			0.669
<b>B. Secondary cohort</b>			
Year 1 values (intercept)			
Ratio of geometric means (I:C)	1.00	(1.02, 0.97)	0.811
Fitted geometric means			
Intensive (ml/min/1.73 m <sup>2</sup> )	122.85	(120.71, 125.02)	
Conventional (ml/min/1.73 m <sup>2</sup> )	123.23	(121.05, 125.44)	
Difference (I-C) (ml/min/1.73 m <sup>2</sup> )	-0.38		
Relative (%) change (slope)			
Intensive	0.26	(-0.14, 0.66)	0.201
Conventional	-0.03	(-0.45, 0.39)	0.874
Difference (I-C)	0.30	(-0.29, 0.88)	0.318
Overall test for treatment group effect (intercept and/or slope), 2df:			0.553

and 122 ml/min/1.73 m<sup>2</sup> in conventionally treated groups in the secondary cohort. In both cohorts combined, 34% of the intensively treated subjects had hyperfiltration (iothalamate clearance > 130 ml/min/1.73 m<sup>2</sup>) at year 3, compared with 39% of conventionally treated subjects (*P* = 0.09). At year 5, 33% of intensively treated subjects had hyperfiltration, compared with 38% of conventionally treated subjects (*P* = 0.36).

#### Blood pressure/hypertension

The appearance of hypertension did not differ between treatment groups in the primary, secondary or combined cohorts. In the primary prevention cohort, 27 subjects in each treatment group developed hypertension for a rate of 1.23 per 100 patient-years and a 17.9% nine year cumulative incidence for the conventionally treated subjects and a rate of 1.34 per 100 patient-years and a 15.9% nine year cumulative incidence for the intensively treated subjects. In the secondary intervention cohort, 57 conventionally treated subjects developed hypertension for a rate of 2.48 per 100 patient-years and a 25.4% nine year cumulative incidence. Fifty intensively treated subjects developed hypertension for a rate of 2.12 per 100 patient-years and a 19.3% cumulative incidence. (*P* = 0.41, compared with conventional)

Analysis of the rate of change of blood pressure showed that

**Table 7.** Iothalamate clearance (ml/min per 1.73 m<sup>2</sup>)

Study time	Group	N	25th Quartile	Median	75th Quartile
Baseline	Primary int	148	114.99	122.70	135.50
	Primary conv	159	113.22	126.29	138.94
	Secondary int	64	117.91	129.80	144.66
	Secondary conv	74	116.58	124.50	140.26
3 Years	Primary int	237	110.22	123.28	137.03
	Primary conv	264	113.89	124.67	139.96
	Secondary int	196	107.02	118.77	135.78
	Secondary conv	218	111.07	122.76	136.77
End (6.5 years) <sup>a</sup>	Primary int	280	114.82	124.62	138.83
	Primary conv	288	115.89	126.34	140.90
	Secondary int	284	109.20	120.98	134.94
	Secondary conv	251	109.31	122.04	135.57

<sup>a</sup> Mean duration of follow-up at study end

within the primary prevention and the secondary intervention cohorts (Table 7), there were no treatment group differences in blood pressure at year 1. Thereafter, in both treatment groups the mean blood pressure increased at a rate of about 0.4% per year in the primary cohort and 0.3% per year in the secondary cohort. These rates of increase were significant within each treatment group, but there was no difference between treatment groups. Because of the previously demonstrated increased incidence of weight gain associated with intensive treatment [8], an additional analysis was performed adjusting for both the percent of ideal body weight at baseline and at each year during follow-up. The results adjusting for body weight over time were comparable to those presented in Table 8.

#### Analysis within subgroups

A number of subject subgroups defined by baseline variables were examined to determine whether the beneficial effect of intensive therapy on the development of microalbuminuria (AER >28 µg/min) was consistent across subgroups (Table 9). The majority of the subject subgroups defined by age, diabetes duration, screening HbA<sub>1c</sub>, baseline smoking status, dietary protein intake, level of retinopathy, neuropathy, the presence or absence of hyperfiltration (C<sub>Cr</sub> <130 or ≥130 ml/min/1.73 m<sup>2</sup>) and other variables experienced a similar beneficial effect of intensive therapy as in the entire cohort. The beneficial effect of intensive treatment on the risk of developing microalbuminuria (AER >28 µg/min) was less among females (18% risk reduction) than among males (57%; *P* = 0.02; data not shown). Excluding women after the onset of pregnancy, the risk reduction was 23% (*P* = 0.06 compared to the risk reduction in men). Further, this difference between the sexes was not seen when the criterion of sustained microalbuminuria was used.

A similar analysis among subgroups was performed examining the relative rate (%) change in AER over time (Table 10). In general, these analyses paralleled the findings in the whole cohort. There was a significant difference between the effect of intensive therapy in males versus females (*P* = 0.03; data not shown), but the difference did not persist if women were excluded from analysis after the onset of pregnancy (*P* = 0.61).

**Table 8.** Summary of regression analysis of ln(mean BP) over time, adjusted for baseline ln(mean BP)

	Estimate	95% C.I.	P <
<b>A. Primary</b>			
Year 1 values (intercept)			
Ratio of geometric means (I:C)	1.01	(1.02, 1.00)	0.277
Fitted geometric means			
Intensive (mm Hg)	85.13	(84.48, 85.79)	
Conventional (mm Hg)	84.62	(84.00, 85.25)	
Difference (I-C) (mm Hg)	0.51		
Relative (%) change (slope)			
Intensive	0.42	(0.20, 0.63)	0.001
Conventional	0.39	(0.18, 0.60)	0.001
Difference (I-C)	0.03	(-0.28, 0.33)	0.867
Overall test for treatment group effect (intercept and/or slope), 2df:			0.353
<b>B. Secondary cohort</b>			
Year 1 values (intercept)			
Ratio of geometric means (I:C)	1.00	(1.01, 0.99)	0.811
Fitted geometric means			
Intensive (mm Hg)	86.87	(86.18, 87.57)	
Conventional (mm Hg)	86.76	(86.05, 87.46)	
Difference (I-C) (mm Hg)	0.12		
Relative (%) change (slope)			
Intensive	0.26	(0.07, 0.46)	0.009
Conventional	0.35	(0.15, 0.55)	0.001
Difference (I-C)	-0.09	(-0.36, 0.19)	0.544
Overall test for treatment group effect (intercept and/or slope), 2df:			0.829

### Discussion

The long-term complications of diabetes appear to develop as a result of an interplay between the abnormal metabolic milieu of diabetes, hemodynamic, and other as yet poorly characterized factors. The DCCT has demonstrated a beneficial effect of intensive diabetes treatment, resulting in a significant improvement in glycemic levels, on the development and progression of the complications of retinopathy, nephropathy and neuropathy [8]. In this paper, we have presented a detailed analysis of the effects of intensive therapy on renal function in the DCCT.

The number of cases developing impaired GFR by either creatinine or iothalamate clearance was too small to permit definitive analysis. Therefore, the development of abnormal levels of AER served as the major outcome. Previous studies have suggested that these may be the earliest, clinically detectable stages of diabetic nephropathy [11-14]. Three specific levels of AER were defined: (1)  $\geq 28 \mu\text{g}/\text{min}$  (40 mg/24 hr) was chosen to represent microalbuminuria, as it is in the middle of the levels chosen by other investigators for this purpose [11-14]; (2)  $\geq 70 \mu\text{g}/\text{min}$  (100 mg/24 hr) was chosen because subjects reaching levels of 100 to 300 mg/24 hr in the Steno follow-up studies had much greater risks of developing clinical albuminuria than did those with levels between 30 and 99 mg/24 hours [26]; and (3)  $\geq 208 \mu\text{g}/\text{min}$  (300 mg/24 hr) was designated as clinical albuminuria comparable to "dipstick positive proteinuria", a recognized stage in the evolution of diabetic nephropathy that progresses to eventual end-stage renal disease in over 90% of affected patients [2-6].

In both the primary prevention and secondary intervention cohorts, intensive therapy reduced the cumulative incidence and overall hazard rates for the development of microalbuminuria.

Intensive therapy reduced the mean adjusted relative risk by 34% and 43% in the two cohorts, respectively, and by 39% for the combined cohorts. Whether this reduction represents absolute prevention or simply a delay in the onset of nephropathy cannot be determined from this study. The numbers of subjects reaching the higher levels of albuminuria were smaller, but again intensive therapy reduced the risks of developing an AER  $> 70 \mu\text{g}/\text{min}$  by 51% and clinical albuminuria by 54% for the combined cohorts. The consistent beneficial effect of intensive therapy by lifetable and longitudinal analyses suggests that intensive therapy will at least delay the onset and progression of nephropathy. Long-term follow-up studies will be needed to examine whether intensive therapy ultimately prevents the development of advanced renal disease in IDDM patients.

The longitudinal analyses of the rates of change of AER showed differences between the two treatment groups within both the primary and secondary cohorts over the duration of the trial; however, there were clearly contrasting patterns between the two cohorts. In the primary prevention cohort, AER was consistently higher with conventional therapy than with intensive therapy. The principal difference was an acute reduction of AER by 15% among intensively treated subjects in the first year, with essentially no further change in this difference in the subsequent years of follow-up. After the first year, the yearly percent change in AER was actually slightly negative in both treatment groups. However, it is important to emphasize that more than 40% of subjects in each treatment group had positive rates of change in AER. The greater number with positive slopes in the conventionally treated group contributed to the greater cumulative incidence of subjects developing microalbuminuria in this treatment group. In the secondary cohort a different pattern emerged with an increase in AER over time of 6.5% per year with conventional therapy versus almost no change in the AER over time with intensive therapy.

The difference between the primary prevention and the secondary intervention cohorts in the rates at which AER progressed in the conventional treatment group may reflect different stages in the natural history of diabetic nephropathy in these separately recruited groups of subjects with different baseline characteristics. On the other hand, intensive therapy was effective in both cohorts, as documented by the absence of an increase in AER over the course of the trial. Thus at these early, but contrasting, stages in the development of diabetic nephropathy, intensive therapy on average prevented the development and slowed the progression of albuminuria. Many cross sectional studies have suggested a correlation between poor diabetic control and risk of development and/or progression of diabetic nephropathy [27-37]. Smaller, randomized studies have compared intensively treated with conventionally treated patients with respect to the development of nephropathy with conflicting results. In the Kroc Study, AER rates fell from baseline in the intensively treated group by 8 months; however, there was no difference in the numbers of intensive versus conventional treatment subjects progressing to higher levels of albuminuria [38]. A similar, randomized one year study with 12 normoalbuminuric patients in each treatment group and achieving a similar HbA<sub>1c</sub> separation as in the DCCT showed no significant changes in AER [39]. The two Steno studies, each of which lasted two years, achieved difference in HbA<sub>1c</sub> between intensively and conventionally treated group of 0.5 and 1.4% and contained 34 and 36 patients, respectively [40, 41]. Only the

**Table 9.** Influence of selected baseline covariates on the relative risk of microalbuminuria (AER  $\geq 28$   $\mu\text{g}/\text{min}$ ) in the combined cohorts with AER  $< 28$   $\mu\text{g}/\text{min}$  at baseline

	Conventional		Intensive		% Reduction in risk <sup>a</sup> (95% C.I.)	Heterogeneity <sup>b</sup> P value
	N	Rate/100 py	N	Rate/100 py		
All patients	694	4.5	671	2.9	39 (21, 52)	—
Demographic						
Gender						
Males	376	4.6	344	2.3	57 (37, 70)	0.06
Females <sup>c</sup>	318	3.8	327	2.9	23 (-15, 49)	
Age						
Adolescents	97	8.6	86	6.2	35 (-7, 60)	0.89
Adults	597	3.8	585	2.4	40 (20, 55)	
IDD						
Duration						
1–2.5 years	238	3.3	206	2.1	28 (-20, 57)	0.92
2.5–5 years	199	3.3	204	2.1	40 (-1, 64)	
5–10 years	134	6.6	122	4.0	50 (18, 69)	
10–15 years	123	6.2	139	4.0	34 (-7, 59)	
HbA1c (quartiles)						
< 7.83%	187	3.0	168	2.4	7 (-63, 47)	0.78
7.83–8.82%	177	3.0	166	2.4	26 (-29, 58)	
8.82–10.10%	156	5.2	178	2.5	59 (33, 75)	
$\geq 10.10\%$	174	7.1	159	4.4	41 (11, 62)	
Clinical and biochemical features						
Dietary protein (% kcal) (tertiles)						
16.66%	224	5.3	224	3.6	15 (2, 56)	0.83
16.66–18.99%	226	4.7	223	2.7	43 (13, 64)	
>18.99%	241	3.4	222	2.4	36 (-3, 60)	
Smoking						
Non-smoker	529	4.2	516	2.9	36 (15, 52)	0.54
Current	165	5.3	155	2.7	48 (13, 69)	
History of UTI						
No	571	4.7	538	2.8	45 (27, 58)	0.09
Yes	121	3.6	132	3.4	7 (-65, 48)	
Retinopathy						
10/10	378	3.4	346	2.2	34 (2, 56)	0.26
20/ $\leq 20$	188	4.7	232	2.9	48 (20, 66)	
30/ $\leq 30$	74	6.4	51	3.4	45 (18, 75)	
40/ $< 40+$	54	9.0	42	8.3	-10 (-112, 43)	
Neuropathy						
No	653	4.2	621	2.8	37 (18, 52)	0.46
Yes	39	8.3	48	3.7	50 (-15, 78)	
Mean blood pressure (tertiles)						
<80 mm Hg	153	4.5	123	3.4	22 (-33, 54)	0.87
80–90 mm Hg	267	4.7	301	2.4	54 (32, 69)	
>90 mm Hg	274	4.2	247	3.2	23 (-14, 48)	
AER						
<10.5 $\mu\text{g}/\text{min}$	481	2.9	480	1.8	41 (15, 59)	0.99
10.5–28 $\mu\text{g}/\text{min}$	213	8.4	191	5.9	37 (11, 56)	
Creatinine clearance $C_{\text{Cr}}$ (quartiles)						
<110 ml.min/1.73m <sup>2</sup>	163	4.0	170	3.3	22 (-29, 53)	0.61
110–126 ml.min/1.73m <sup>2</sup>	184	4.6	157	1.9	58 (25, 76)	
126–143 ml.min/1.73m <sup>2</sup>	174	4.3	180	2.8	43 (5, 65)	
>143 ml.min/1.73m <sup>2</sup>	173	4.9	164	3.6	33 (-6, 57)	
Hyperfiltration						
$C_{\text{Cr}} < 130$ ml/min/1.73 m <sup>2</sup>	390	4.4	382	2.6	41 (17, 58)	0.64
$C_{\text{Cr}} \geq 130$ ml.min/1.73 m <sup>2</sup>	304	4.6	289	3.3	37 (105, 56)	
LDL cholesterol (quartile)						
< 91 mg/dl	185	4.6	158	3.2	31 (-12, 57)	0.96
91–107 mg/dl	156	5.1	180	2.7	52 (21, 71)	
107–127 mg/dl	173	4.6	166	3.1	32 (-12, 58)	
>127 mg/dl	179	3.7	167	2.5	38 (-5, 64)	
Familial						
Family history of hypertension						
No	306	4.0	292	2.8	29 (-4, 52)	0.37
Yes	388	4.9	379	2.9	44 (23, 60)	

<sup>a</sup> Percentage reduction in risk of intensive compared with conventional treatment calculated from a proportional hazards regression model adjusted for baseline AER values and stratified by primary and secondary cohorts.

<sup>b</sup> Test of equality of risk reductions among categories of the covariate obtained from a test of interaction between treatment groups and the covariate in a proportional hazards regression model.

<sup>c</sup> In order to adjust for the effects of pregnancy on AER levels women who are pregnant are censored or ignored beyond the time of pregnancy or in the conventional group beyond the time of deviation to intensive therapy for purposes of preconception glucose control.

**Table 10.** Influence of selected baseline characteristics on the treatment group effects on relative change in AER over time in the secondary cohort

	N	Relative (%) change in AER/year			P value
		Conventional	Intensive	Difference (I-C)	
All patients	715	6.46	-0.25	-6.72 (-9.60, -3.83)	≤ 0.001
Gender					
Male	383	8.76	0.44	-8.32 (-12.34, -4.29)	0.0001
Female	332	5.24	-1.43	-6.67 (-11.44, -1.91)	0.007
Treatment by subgroup interaction					0.606
Baseline creatinine clearance					
< 130 ml/mn/1.73 m <sup>2</sup>	389	7.40	-1.36	-8.77 (-12.79, -4.74)	< 0.0001
≥ 130 ml/mn/1.73 m <sup>2</sup>	326	5.37	1.09	-4.28 (-8.38, -0.17)	0.041
Treatment by subgroup interaction					0.879
Baseline IDDM duration					
< 5 years	145	3.13	1.84	-1.29 (-6.65, 4.07)	0.638
≥ 5 years	570	7.42	-0.73	-8.14 (-11.49, -4.80)	< 0.0001
Treatment by subgroup interaction					0.540
Baseline AER					
< 28 μg/min	642	5.90	-0.60	-6.50 (-9.50, -3.50)	< 0.0001
≥ 28 μg/min	73	11.02	2.47	-8.55 (-18.34, 1.24)	0.087
Treatment by subgroup interaction					0.089

second study showed a significant effect of treatment on AER. In the conventional treatment group, the fractional albumin clearance more than doubled and 5 of the 18 patients progressed to AER >300 mg/24 hr (208 μg/min). In the intensive treatment group, the fractional albumin clearance did not change significantly and none of the 18 patients progressed to AER >300 mg/24 hr (208 μg/min) [41]. The change in fractional clearance in the conventional group was significantly different from that in the intensive group. Also, the slopes of the bimonthly AER values over two years among patients in the conventional group were significantly higher than those in the intensive group. A meta-analysis of the combined studies eight (Steno 1) and five (Steno 2) years after their completion, but according to the original treatment assignment, showed that intensive treatment decreased the number of subjects progressing to an AER level >300 mg/24 hr (208 μg/min) only in patients with baseline AER levels >100 mg/24 hr [27].

In the Oslo study, patients were treated intensively with either continuous subcutaneous insulin infusion via a pump or with multiple insulin injections, or with conventional treatment (15 in each of three groups) and were followed prospectively over a 43 to 47 month period. There were no significant differences in renal outcome among the groups at study end [42]. An analysis of data obtained from the patients when they no longer were required to remain in their assigned treatment groups, similar to that performed for the Steno studies, showed no differences in the levels of AER in the groups when they were analyzed according to their original "intention to treat" classification [43].

The Stockholm Diabetes Study contained the largest number of patients (N = 102) followed for the longest period of time (5 to 7.5 years; 91 subjects completed 7.5 years) prior to the DCCT [44]. It maintained a 1.4% difference in HbA<sub>1c</sub> between treatment groups and documented a significant treatment group effect on the development of AER >200 μg/min.

Inspection of Figures 2 through 4 helps to explain the negative findings in some of these previous studies. It is clear that differences between the treatment groups with respect to development of AER levels >28 and 70 μg/min did not appear until after three years of therapy. A difference between the treatment

groups in the development of clinical albuminuria only appeared after five years of treatment. Most of the previous studies were not carried out long enough or with sufficient numbers of subjects to detect treatment group differences. The beneficial effect of intensive therapy in the relatively brief Steno 2 study may be explained by the fact that the average of AER measured in two 24-hour urine collections performed every two months was used as the basis for analysis. The repeated, frequent measurements may have reduced inpatient variability. Also, a relatively large number of subjects entered that study with AER >70 μg/min (but < 208 μg/min), a point at which intensive therapy may be particularly effective. Although the decrease in the development of clinical albuminuria in the 73 patients who entered with AER ≥28 μg/min but less than 139 μg/min was not significant in the DCCT, an analysis of the slopes of AER over time revealed a reduction in the mean slope with intensive versus conventional patients similar in magnitude among these 73 patients (8.55%/year, P = 0.09) to the difference in 642 secondary cohort patients (6.5%/year, P < 0.01) who entered with AER <28 μg/min.

The large number of patients included in the DCCT permits examination of consistency of the beneficial effect of intensive therapy in subgroups of patients defined by baseline characteristics. These analyses show a similar reduction in risk for development of microalbuminuria (≥28 μg/min) in the intensively treated group, irrespective of age, duration of diabetes, family history of hypertension, smoking status, presence of neuropathy, severity of retinopathy, and initial levels of HbA<sub>1c</sub>, blood pressure, AER, creatinine clearance, and LDL-cholesterol.

The nearly significant difference of treatment effect in female as contrasted with male subjects could relate to the well described increased risk of renal disease in male patients with IDDM [45, 46]. Therefore, diabetic men may more readily benefit from an equivalent success in achieving the goals of intensive therapy. Stated alternatively, for a given reduction in hemoglobin A<sub>1c</sub> the male participants may experience a greater reduction in risk for reaching the renal benchmarks established by the DCCT and possibly for end-stage renal disease.

In summary, intensive treatment reduced the risk of developing any microalbuminuria by 39% and clinical albuminuria by 54%. In

**Table A.** Linear trend of AER over time: Model coefficients and variance component estimates within each cohort

	Primary cohort			Secondary cohort		
	Est.	SE	P<	Est.	SE	P<
<b>Coefficients</b>						
$\bar{\alpha}_0$ : Intercept (yr 1)	1.305	0.055	<0.001	1.008	0.065	0.001
$\alpha_1$ : Group (I = -1, C = +1)	-0.080	0.020	<0.001	-0.018	0.024	0.46
$\bar{\beta}_0$ : Time (yr)	-0.0092	0.006	0.13	0.031	0.007	0.001
$\beta_1$ : Group $\times$ Time	-0.0040	0.006	0.52	-0.034	0.007	0.001
$\delta$ : Baseline (ln $\mu\text{g}/\text{min}$ )	0.346	0.027	<0.001	0.543	0.027	0.001
<b>Variance components</b>						
$\sigma_\alpha^2$ : Intercept ( $\alpha$ )	0.138	0.016	<0.001	0.259	0.022	0.001
$\sigma_\beta^2$ : Time ( $\beta$ )	0.0075	0.0013	<0.001	0.024	0.0020	0.001
$\sigma_{\alpha\beta}$ : $\alpha\beta$	0.0011	0.0036	0.77	0.0066	0.0049	0.18
$\sigma_\epsilon^2$ : Within subjects	0.303	0.0080	<0.001	0.319	0.0077	0.001

**Table B.a.** The secondary cohort: Quadratic model of AER over time: Coefficients and variance component estimates

	Est.	SE	P<
<b>Coefficients</b>			
$\bar{\alpha}_0$ : Intercept (year 1)	0.976	0.065	0.001
$\alpha_1$ : Group (I = -1, C = +1)	-0.015	0.026	0.56
$\bar{\beta}_0$ : Linear trend in time (years)	0.072	0.015	0.001
$\beta_1$ : Group $\times$ linear trend	-0.036	0.015	0.019
$\delta_0$ : Quadratic trend (yr) <sup>2</sup>	-0.0065	0.0021	0.002
$\delta_1$ : Group $\times$ quadratic trend	0.0002	0.0021	0.92
$\nu$ : Baseline (ln mg/24 hr)	0.540	0.027	0.001
<b>Variance components</b>			
$\sigma_\alpha^2$ : Intercept ( $\alpha$ )	0.253	0.025	0.001
$\sigma_\beta^2$ : Time linear ( $\beta$ )	0.00040	0.0015	0.80
$\alpha_\beta^2$ : Time quadratic ( $\delta$ )	0.00055	0.00014	0.001
$\sigma_\epsilon^2$ : Within subjects	0.3035	0.0078	0.001

subjects in the secondary intervention cohort, with longer duration and higher baseline AER levels, the rate of increase in AER in the intensive treatment group was virtually zero compared with 6.5%/year with conventional therapy. Whether these changes represent only a retardation of development and progression or represent prevention of diabetic nephropathy in a subset of patients can only be determined by a prolonged follow-up of these patients. These data, coupled with the benefits also shown for diabetic retinopathy and neuropathy, outweigh the risk of severe adverse effects with intensive treatment. [8] They constitute strong support for the institution of intensive therapy in most patients with IDDM unless there are specific factors that would alter this benefit:risk ratio.

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**Appendix**

*Random effects longitudinal models of albumin excretion rate*

*Regression models*

Longitudinal growth-curve analyses [23, 24] were performed separately within each treatment group, and for both groups combined. Separate analyses were performed using only a linear trend over time, and then using both a linear and a quadratic trend. Analyses were performed using the program 5V of BMDP [26] and the estimates obtained using maximum likelihood [24] are presented. In all cases, analyses using REML [23] yield nearly identical results.

The linear trend statistical models employed in these analyses are of the following form. The intensive and conventional treatment groups are designated as  $j' = I$  or  $C$ , respectively, and  $i$  refers to an individual subject in either group. Then let  $X_{ij}$  refer to the natural log (ln) of the baseline measure obtained prior to randomization, and  $Y_{ijt}$  refer to the ln of the followup measure at time  $t$ , ( $t = 1, \dots, 9$ ) for the  $i$ -th patient in the  $j$ -th group.

**Table B.b.** The secondary cohort: Summary of quadratic trend regression analysis of AER over time

	Estimate	95% C.I.	P<
<b>Year 1 Values (intercept)</b>			
Ratio of geometric means (I:C)	0.97	(1.07, 0.88)	0.556
Fitted geometric means	8.72	(8.12, 9.35)	
Intensive ( $\mu\text{g}/\text{min}$ )			
Conventional ( $\mu\text{g}/\text{min}$ )	8.98	(8.36, 9.65)	
Difference (I-C) ( $\mu\text{g}/\text{min}$ )	-0.27		
<b>Relative (%) change (slope)</b>			
Intensive	3.66	(-0.52, 7.83)	0.086
Conventional	10.80	(6.55, 15.05)	0.001
Difference (I-C)	-7.14	(-13.10, -1.19)	0.019
<b>Derivative of relative (%) change</b>			
Intensive	-0.63	(-1.20, -0.05)	0.033
Conventional	-0.67	(-1.26, -0.08)	0.033
Difference (I-C)	0.04	(-0.78, 0.86)	0.920
Overall test for treatment group effect (intercept and/or slope), 3df:			0.001

*Separate group-specific models.* Within each group separately ( $j = I$  or  $C$ ), the group specific linear trend model is of the following form:

$$Y_{ijt} = \alpha_{ij} + \beta_{ij}(t - 1) + \gamma_j X_{ij} + \epsilon_{ijt} \tag{1}$$

where the errors,  $\epsilon_{ijt}$ , are assumed to be independently normally distributed with mean zero and variance  $\sigma_\epsilon^2$ . This model has two structural components. For the  $i$ -th subject (in the  $j$ -th group) this model states that the expected value of the ln (measure) at any time  $t$ ,  $E(Y_{ijt})$ , is a linear function of the baseline value  $X_{ij}$  with slope  $\gamma_j$  for the members of the  $j$ -th group. This provides an "adjustment" for the baseline value. The model also states that  $E(Y_{ijt})$  is a linear function of time for each subject through a subject-specific intercept  $\alpha_{ij}$  and slope  $\beta_{ij}$  for the  $i$ -th subject in the  $j$ -th group.

Within the  $j$ -th group, however, the  $\alpha$ s and  $\beta$ s themselves are also assumed to be jointly normally distributed with a mean intercept  $\bar{\alpha}_j$  and a mean slope  $\bar{\beta}_j$  for all the subjects in that group. Since time enters the model as  $(t - 1)$ , then the average intercept  $\bar{\alpha}_j$  reflects the acute effect of that treatment on the levels at year 1, and the average slope  $\bar{\beta}_j$  reflects the long-term trend in that group beyond the first year. A test of the difference between these average group intercepts ( $\bar{\alpha}_I$  vs.  $\bar{\alpha}_C$ ) is a test of a difference in the acute (short-term) effects of treatment. Likewise, a test of the difference between these average slopes ( $\bar{\beta}_I$  vs.  $\bar{\beta}_C$ ) is a test of a difference in the long-term effects of treatment.

Another feature of these models is that within each group, the subject-specific intercepts and slopes are randomly distributed with variances  $\sigma_{\alpha_j}^2$  and  $\sigma_{\beta_j}^2$ , respectively, and covariance  $\sigma_{\alpha\beta_j}$ . These variance

**Table C.a.** Linear trend model of AER over time: Coefficients and variance component estimates within the primary cohort

	Intensive			Conventional			I vs. C
	Est.	SE	P<	Est.	SE	P<	P<
<b>Coefficients</b>							
$\alpha$ : Intercept (yr 1)	1.215	0.069	<0.001	1.392	0.083	<0.001	0.16
$\beta$ : Time (yr)	-0.012	0.0081	0.15	-0.0059	0.0088	0.51	0.62
$\nu$ : Baseline (ln mg/24 h)	0.350	0.034	<0.001	0.343	0.040	<0.001	0.90
<b>Variance components</b>							
$\sigma_{\alpha}^2$ : Intercept ( $\alpha$ )	0.768	0.0173	<0.001	0.1939	0.0264	<0.001	<0.001
$\sigma_{\beta}^2$ : Time ( $\beta$ )	0.0055	0.0015	<0.001	0.0091	0.0020	<0.001	0.15
$\sigma_{\alpha\beta}$ : $\alpha\beta$	0.0050	0.0042	0.23	-0.0020	0.0058	0.74	0.33
$\sigma_{\epsilon}^2$ : Within subjects	0.2868	0.0109	<0.001	0.3190	0.0117	<0.001	0.05

**Table C.b.** Linear trend model of AER over time: Coefficients and variance component estimates within the secondary cohort

	Intensive			Conventional			I vs. C
	Est.	SE	P<	Est.	SE	P<	P<
<b>Coefficients</b>							
$\alpha$ : Intercept (yr 1)	0.952	0.090	<0.001	1.067	0.092	<0.001	0.39
$\beta$ : Time (yr)	-0.0019	0.0081	0.82	0.063	0.012	<0.001	<0.001
$\nu$ : Baseline (ln mg/24 h)	0.559	0.037	<0.001	0.525	0.039	<0.001	0.53
<b>Variance components</b>							
$\sigma_{\alpha}^2$ : Intercept ( $\alpha$ )	0.2779	0.0312	<0.001	0.2410	0.0310	<0.001	0.41
$\sigma_{\beta}^2$ : Time ( $\beta$ )	0.0115	0.0017	<0.001	0.0371	0.0040	<0.001	<0.001
$\sigma_{\alpha\beta}$ : $\alpha\beta$	0.0045	0.0055	0.41	0.0087	0.0080	0.28	0.67
$\sigma_{\epsilon}^2$ : Within subjects	0.2960	0.0100	<0.001	0.3441	0.0119	<0.001	0.002

components reflect the degree of variation of the intercepts and slopes within the j-th group. Within a treatment group, the method of maximum likelihood provides estimates of the coefficients  $\bar{\alpha}_j$ ,  $\bar{\beta}_j$ , and  $\gamma_j$ , and of the variance components  $\sigma_{\alpha_j}^2$ ,  $\sigma_{\beta_j}^2$ ,  $\sigma_{\alpha\beta_j}$  and  $\sigma_{\epsilon_j}^2$ , and also provides estimates of the large-sample standard errors of each. This allows tests of the significance of differences between groups.

*A single model with treatment group effects.* Another, more general approach is to assume that the variance components are equal in the two treatment groups and to then fit a single model which incorporates treatment group effects in the coefficients. Here we let i designate a subject from both treatment groups combined. For the i-th subject, an additional variable  $G_i$  is used to designate the treatment group of that subject, where  $G_i = +1$  if I, or  $-1$  if C. The overall model then is of the form

$$Y_{it} = \alpha_{i0} + \alpha_i G_i + \beta_{i0}(t - 1) + \beta_i G_i(t - 1) + \gamma \bar{X}_i + \epsilon_{it} \quad (2)$$

As before, the intercepts  $\alpha_{i0}$  and slopes  $\beta_{i0}$  are assumed to be randomly distributed in the population with means  $\bar{\alpha}_0$  and  $\bar{\beta}_0$ , and variance components  $\sigma_{\alpha_0}^2$ ,  $\sigma_{\beta_0}^2$ , and covariance  $\sigma_{\alpha\beta_0}$ ; and the errors  $\epsilon_{it}$  are independently distributed with mean zero and variance  $\sigma_{\epsilon}^2$ . Because the  $G_i = \pm 1$ , then the mean intercept and slopes in the two groups are expressed as:

	Intercept	Slope	
Intensive:	$\alpha_I = \bar{\alpha}_0 + \alpha_1$	$\beta_I = \bar{\beta}_0 + \beta_1$	(3)
Conventional:	$\alpha_C = \bar{\alpha}_0 - \alpha_1$	$\beta_C = \bar{\beta}_0 - \beta_1$	

Thus, a test that  $\alpha_1 = 0$  is equivalent to a test that  $\bar{\alpha}_I = \bar{\alpha}_C$ ; and a test that  $\beta_1 = 0$  is equivalent to a test that  $\bar{\beta}_I = \bar{\beta}_C$ . Each of these hypotheses can be tested using a 1 df standard normal (or chi square) Wald test based on the estimated coefficients and their standard errors. In addition, an overall test of treatment group difference in either the intercepts and/or the slopes (a test that both  $\alpha_1$  and  $\beta_1$  equal zero) is provided by a 2 df Wald chi square test obtained as a contrast among the estimates and their covariance matrix.

*Albumin excretion rate*

*Linear trend models.* Appendix Table A presents the fit of the model (2) to the ln(AER) measures separately within the primary and the secondary cohorts. This model was used as the basis for Table 4. Because the

dependent variable is the ln(AER), then  $e^{2\alpha_1}$  is the ratio of the geometric means of intensive to conventional treatment (I:C), and a test that  $\alpha_1$  is zero is equivalent to a test that this ratio is one. Confidence limits on the ratio can be obtained by likewise transforming the confidence limits on  $\alpha_1$ . Under the model (2), the geometric mean AER in each group at year 1, where  $t = 1$  and  $(t - 1) = 0$ , are obtained as

$$e^{(\bar{\alpha}_0 + \alpha_1 G + \gamma \bar{X})}$$

where  $G = +1$  if I, or  $-1$  if C; and where  $\bar{X}$  is the overall mean of the baseline ln(AER). The exponent is the mean of the ln(AER) under the model and its standard error can be obtained from the variance of the linear combination of the parameter estimates. This then provides confidence limits on the mean of the ln(AER). Transforming these confidence limits yields asymmetric confidence limits on these geometric means. The difference between the estimated geometric means is also presented in Table 4.

To describe the long-term trend over time, the coefficient  $\beta$  in the above models represents the change in the mean of Y per unit change in t, that is, per year. Since Y represents the ln(AER), then  $e^{\beta}$  reflects the relative change over time, and  $(e^{\beta} - 1) * 100$  is the percent change per year. For values of  $\beta$  relatively close to zero, this expression is approximately equal to simply  $\beta * 100$ . Thus, the coefficient itself can be interpreted (approximately) as the relative change in AER ( $\mu\text{g}/\text{min}$ ) per year. In the model (2), the slopes in each group are obtained from (3), and the variance of the estimated slopes is obtained from the variance of the respective linear combinations of the estimates of  $\bar{\beta}_0$  and  $\beta_1$ , and likewise that for the difference between groups. A test that  $\beta_1$  is zero then is a test of no treatment group differences in long-term trend.

Overall, the groups may differ either in their intercepts ( $\alpha_1$ ) or in their slopes ( $\beta_1$ ): An overall test of treatment group effect, therefore, is obtained as a 2 df test that both of these coefficients equal zero versus the alternative that either or both are different from zero. The p-value for this test is presented in Table 4.

*Quadratic trend models.* Separately within the primary and the secondary cohorts, quadratic trend analyses were also performed in which quadratic time effect terms  $\delta_{i0}(t - 1)^2$  and  $\delta_{i1}G_i(t - 1)^2$  were added to model (2), with a corresponding mean and variance component,  $\bar{\delta}_{i0}$  and  $\sigma_{\delta}^2$ , respectively, for the random effect. Quadratic effects were only significant in the model for the secondary cohort; this model is presented



in Appendix Table B. Table B.a presents the fitted model coefficients and variance components, and Table B.b presents the model in terms of the rate of change over time. In this model, the quadratic effect corresponds to the effect of the square of time on the  $\ln(\text{AER})$ , thus describing the second derivative of the curve relating AER to time. Thus, the quadratic effect can be interpreted as the derivative of the relative percent change in AER per year, or as the rate of change in the slope over time.

As in Table A, there is a significant linear trend and a significant treatment group difference in this trend. In addition, there was a significant overall quadratic trend ( $\delta_{10}$ ) but the group by quadratic trend interaction ( $\delta_{11}$ ) was not significant, indicating no significant difference between groups (Table B.a). The respective derivatives of the percent change per year are presented in Table B.b. In each group, these derivatives are negative and are nominally significant ( $P < 0.035$  for each). Since a positive linear trend in the  $\ln(\text{AER})$  represents a convex trend in AER, then a negative quadratic trend will dampen the rate of increase in AER within both treatment groups. Thus, in the intensive group, for example, the percent change during the second year ( $t = 2$ ) is  $3.66 - (0.63)(2 - 1) = 3.03\%$  change from the value at year 1 to that at year 2.

*Linear trend components of variance.* Appendix Table C presents the fit of the group-specific linear trend models (1) separately for the intensive and conventional treatment groups within the primary cohort (Table C.a) and the secondary cohort (Table C.b). Also presented are tests of differences between groups for the various model parameters using a large sample Z-test (two-sided). Within the primary cohort, the only additional finding compared to Appendix Table A is that the variance component for the intercepts ( $\sigma_a^2$ ) differs significantly between groups, the variance among conventional group patients being threefold greater than that among intensive group patients. Thus, a greater fraction of conventional than intensive group patients had a "high" intercept. Also the error variance ( $\sigma_e^2$ ) was significantly higher (at  $P < 0.05$ ) among conventional than intensive group patients, indicating a better fit of the model among the latter.

Within the secondary cohort (Table C.b), the trend coefficients are significantly different, as was the case in Table 3. Also, the variance of the slopes ( $\sigma_b^2$ ) is threefold greater among conventional than intensive group patients ( $P < 0.001$ ). Thus, the distribution of slopes among conventional subjects not only has a greater mean, but also a greater variance (spread) than that of intensive subjects. This indicates that a higher fraction of conventional subjects showed a progression in AER.

## References

- ROSENSTOCK J, RASKIN P: Early diabetic nephropathy: Assessment and potential therapeutic interventions. *Diabetes Care* 9:529-545, 1986
- SELBY JV, FITZSIMMONS SC, NEWMAN JM, KATZ PP, SEPE S, SHOWSTACK J: The natural history and epidemiology of diabetic nephropathy. Implications for prevention and control. *JAMA* 263:1954-1960, 1990
- NOTH RH, KROLEWSKI AS, KAYSAN GA, MEYER TW, SCHAMBELAN M: Diabetic Nephropathy: Hemodynamic basis and implications for disease management. *Ann Intern Med* 110:795-813, 1989
- 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
- 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, WARRAM JH, CHRISTLIEB AR, BUSICK EJ, KAHN CR: The changing natural history of nephropathy in Type 1 diabetes. *Am J Med* 78:785-797, 1985
- THE DCCT RESEARCH GROUP: The Diabetes Control and Complications Trial (DCCT): Design and methodologic considerations for the feasibility phase. *Diabetes* 35:530-545, 1986
- THE 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* 329:977-86, 1993
- THE DCCT RESEARCH GROUP: Baseline analysis of renal function in the Diabetes Control and Complications Trial. *Kidney Int* 43:668-674, 1993
- FRIEDEWALD WT, LEVY RI, FREDERICKSON DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 18:499-502, 1972
- PARVING HH, OXENBOLL B, SVENDSEN PA, CHRISTIANSEN JS, ANDERSEN AR: Early detection of patients at risk of developing diabetic nephropathy. A longitudinal study of urinary albumin excretion. *Acta Endocrinol* 100:550-555, 1982
- VIBERTI GC, HILL RD, JARRETT RJ, ARGYROPOULOS A, MAHMUD U, KEEN H: Micro albuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet* 1:1430-1432, 1982
- MATHIESEN ER, OXENBOLL B, JOHANSEN K, SVENDSEN PA, DECKERT T: Incipient nephropathy in Type 1 (insulin-dependent diabetes). *Diabetologia* 26:406-410, 1984
- MOGENSEN CE, CHRISTENSEN CK: Predicting diabetic nephropathy in insulin-dependent patients. *N Engl J Med* 311:89-93, 1984
- THE DCCT RESEARCH GROUP: Color photography vs fluorescein angiography in the detection of diabetic retinopathy in the Diabetes Control and Complications Trial. *Arch Ophthalmol* 105:1344-1351, 1987
- LEVY AS, GREENE T, SCHLUCHTER MD, CLEARY PA, TESCHAN PE, LORENZ RA, MOLITCH ME, MITCH WE, SIEBERT C, HALL PM, STEFFES MW, FOR THE MODIFICATION OF DIET IN RENAL DISEASE STUDY GROUP AND THE DIABETES CONTROL AND COMPLICATIONS TRIAL RESEARCH GROUP: Glomerular filtration rate measurements in clinical trials. *JASN* 4:1159-1171, 1993
- LEVY AS: Nephrology Forum: Measurement of renal function in chronic renal disease. *Kidney Int* 38:167-184, 1990
- LEE ET: *Statistical Methods for Survival Data Analysis*. Belmont, Lifetime Learning Publications, Inc., 1980, pp 88-92, 127-129, 306-312
- TYGSTRUP N, LACHIN JM, JUHL E (EDITORS): *The Randomized Clinical Trial and Therapeutic Decisions*, New York, Marcel Dekker, 1982, p 174
- SNEDECOR GW, COCHRAN WG: *Statistical Methods* (6th ed), Ames, Iowa State University Press, 1980
- WEI LJ, LACHIN JM: Two-sample asymptotically distribution-free tests for incomplete multivariate observations. *J Am Stat Assoc* 79:653-661, 1984
- LACHIN JM: Some large sample distribution-free estimators and tests for multivariate partially incomplete observations from two populations. *Stat Med* 11:1151-1170, 1992
- LAIRD NM, WARE JH: Random-effects models for longitudinal data. *Biometrics* 38:963-974, 1982
- JENNRICH RI, SCHLUCHTER MD: Unbalanced repeated-measures models with structured covariance matrices. *Biometrics* 42:805-820, 1986
- BLOOMQVIST N: On the relation between change and initial value. *J Am Stat Assn* 72:746-49, 1977
- DIXON WJ, BROWN MB, ENGELMAN L, HILL MA, JENNRICH, RI: *BMDP Statistical Software Manual - Volume 2* University of California Press, Los Angeles, 1988
- FELDT-RASMUSSEN B, MATHIESEN ER, JENSEN T, LAURITZEN T, DECKERT T: Effect of improved metabolic control on loss of kidney function in Type 1 (insulin-dependent) diabetic patients: An update of the Steno studies. *Diabetologia* 34:164-170, 1991
- CHAZAN BI, BALODIMOS MC, RYAN JR, MARBLE A: Twenty-five to forty-five years of diabetes with and without vascular complications. *Diabetologia* 6:565-569, 1970
- PIRART J: Diabetes and its degenerative complications: A prospective study of 4400 patients observed between 1947 and 1973. *Diabetes Care* 1:168-188, 252-263, 1978
- DECKERT T, POULSEN JE, LARSEN M: Prognosis of diabetics with diabetes onset before age of thirty one. II. Factors influencing the prognosis. *Diabetologia* 14:371-377, 1978
- VIBERTI GC, MACKINTOSH D, BILOUS RW, PICKUP JC, KEEN H: Proteinuria in diabetes mellitus: Role of spontaneous and experimental variations of glycaemia. *Kidney Int* 21:714-720, 1982
- WISEMAN M, VIBERTI G, MACKINTOSH D, JARRETT RJ, KEEN H: Glycaemia, arterial pressure and microalbuminuria in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 26:401-405, 1984
- BORCH-JOHNSEN K, NISSEN H, SALLING N, HENRIKSEN E, KREINER S, DECKERT T, NERUP J: The natural history of insulin-dependent diabetes in Denmark. 2. Long-term survival: Who and why. *Diabetic Med* 4:211-216, 1987

34. NYBERG G, BLOHME G, NORDEN G: Impact of metabolic control in progression of clinical diabetic nephropathy. *Diabetologia* 30:82–86, 1987
35. CHASE HP, JACKSON WE, HOOPS SL, COCKERHAM RS, ARCHER PG, O'BRIEN D: Glucose control and the renal and retinal complications of insulin-dependent diabetes. *JAMA* 261:1155–1160, 1989
36. ORCHARD TJ, DORMAN JS, MASER RE, BECKER DJ, ELLIS D, LA-ORTE RE, KULLER LH, WOLFSON SK JR, DRASH AL: Factors associated with avoidance of severe complications after 25 years of IDDM. Pittsburgh Epidemiology of Diabetes Complications Study I. *Diabetes Care* 13:741–747, 1990
37. MCCANCE DR, HADDEN DR, ATKINSON AB, JOHNSTON H, KENNEDY L: The relationship between long-term glycaemic control and diabetic nephropathy. *Quart J Med NS* 82:53–61, 1992
38. THE KROC COLLABORATIVE STUDY GROUP: Blood glucose control and the evolution of diabetic retinopathy and albuminuria. A preliminary multicenter trial. *N Engl J Med* 311:365–372, 1984
39. BECK-NIELSEN H, RICHENSEN B, MOGENSEN CE, OLSEN T, EHLERS N, NIELSEN CB, CHARLES P: Effect of insulin pump treatment for one year on renal function and retinal morphology in patients with IDDM. *Diabetes Care* 8:585–589, 1985
40. LAURITZEN T, FROST-LARSEN K, LARSEN HW, DECKERT T FOR THE STENO STUDY GROUP: Two years' experience with continuous subcutaneous insulin infusion in relation to retinopathy and neuropathy. *Diabetes* 34 (Suppl 3):74–79, 1985
41. FELDT-RASMUSSEN B, MATHIESEN ER, DECKERT T: Effect of two years of strict metabolic control on progression of incipient nephropathy in insulin-dependent diabetes. *Lancet* 2:1300–1304, 1986
42. DAHL-JØRGENSEN K, HANSEN KF, KIERULF P, BJØRO T, SANDVIK L, AAGENÆS Ø: Reduction of urinary albumin excretion after 4 years of continuous subcutaneous insulin infusion in insulin-dependent diabetes mellitus. The Oslo Study. *Acta Endocrinol (Copenh)* 117:19–25, 1988
43. DAHL-JØRGENSEN, BJØRO T, KIERULF P, SANDVIK L, BANSTAD HJ, HANSEN KF: Long-term glycaemic control and kidney function in insulin-dependent diabetes mellitus. *Kidney Int* 41:920–923, 1992
44. REICHARD P, NILSSON B-Y, 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
45. COWIE CC, PORT FK, WOLFE RA, SAVAGE PJ, MALL PP, HAWTHORNE VM: Disparities in incidence of diabetic end-stage renal disease according to race and type of diabetes. *N Engl J Med* 321:1074–1079, 1989
46. *U. S. Renal Data System, USRDS 1994 Annual Data Report*, The National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, June 1994