

# Risk of Nephropathy Can Be Detected Before the Onset of Microalbuminuria During the Early Years After Diagnosis of Type 1 Diabetes

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**OBJECTIVE** — The early detection of a rise in albumin excretion within the normal range could permit early intervention to prevent the development of microalbuminuria (MA) in genetically susceptible subjects with type 1 diabetes. In the Oxford Regional Prospective Study, we prospectively examined urine albumin excretion during the first years after diagnosis of childhood type 1 diabetes.

**RESEARCH DESIGN AND METHODS** — Between 1986 and 1995, 511 subjects aged <16 years were recruited at diagnosis and followed for a median of 6 years (range 1–14). In 78 subjects (designated cases), an annual assessment of the albumin-to-creatinine ratio (ACR) in three morning first-void urine samples detected MA (males: ACR  $\geq 3.5$  mg/mmol, females: ACR  $\geq 4.0$  mg/mmol in two of three urine samples). In 63 of these subjects and 396 normoalbuminuric diabetic control subjects, rates of change of the ACR were calculated as the slope of the ACR over diabetes duration.

**RESULTS** — The baseline ACR (median [interquartile (IQ) range]), as measured at 1–2.5 years' duration of diabetes, was higher in microalbuminuric subjects than in the normoalbuminuric subjects (1.0 mg/mmol [0.6–2.1],  $n = 52$ , vs. 0.8 mg/mmol [0.6–1.2],  $n = 303$ ;  $P = 0.02$ ). The rate of increase of the ACR in the years before the onset of MA was higher in the microalbuminuric subjects than in the normoalbuminuric subjects (70% per year [37–149],  $n = 63$ , vs. 1% per year [–9 to 13],  $n = 396$ ;  $P < 0.001$ ). The mean HbA<sub>1c</sub> level after the onset of puberty was weakly correlated with the rate of change of the ACR ( $r = 0.11$ ,  $P = 0.024$ ,  $n = 418$ ).

**CONCLUSIONS** — Higher levels of ACR within the first 2 years after diagnosis and a significantly higher rate of increase of the ACR within the first 5 years from diagnosis can be detected in subjects who subsequently develop MA. HbA<sub>1c</sub> is a determinant of risk for MA, but pubertal factors have a greater effect on rates of progression of urine albumin excretion during adolescence in this cohort.

*Diabetes Care* 23:1811–1815, 2000

Diabetic nephropathy is associated with high morbidity and mortality in type 1 diabetes (1). Nevertheless, only a proportion (30–50%) of subjects with type 1 diabetes is at risk for nephropathy (1), and genetic factors are thought to be important in determining susceptibility (2). During adolescence, HbA<sub>1c</sub>, sex, and puberty (3,4) are also important determinants of the risk for incipient nephropathy (microalbuminuria [MA]). However, it may be possible to detect individuals at risk at an earlier stage in the pathogenesis of nephropathy. It has been established that, in adults, levels of urine albumin excretion within the normal range are higher in individuals who subsequently develop MA (5,6). Furthermore, a progressive rise in urine albumin excretion within the normal range was detected before the onset of incipient nephropathy in adults (7). However, there is little longitudinal data on early changes in urine albumin excretion during childhood and adolescence, and there is no information regarding the early changes in subjects who were followed from the diagnosis of type 1 diabetes (8).

We examined the early detection and determinants of changes in urine albumin excretion from diagnosis of type 1 diabetes in a large cohort of children and adolescents in the Oxford Regional Prospective Study (ORPS).

## RESEARCH DESIGN AND METHODS

Eligible subjects aged <16 years within a geographically defined region were recruited at diagnosis of type 1 diabetes and were followed annually from diagnosis. Case ascertainment, recruitment, and the drop-out rate were reported elsewhere (3). Ethical approval was obtained from district ethics committees. Written consent was obtained from parents, and children were asked to give assent before the study. The study design and methods used in the ORPS have been described previously (3). Briefly, subjects were asked annually to provide three consecutive first-void morning urine specimens for the mea-

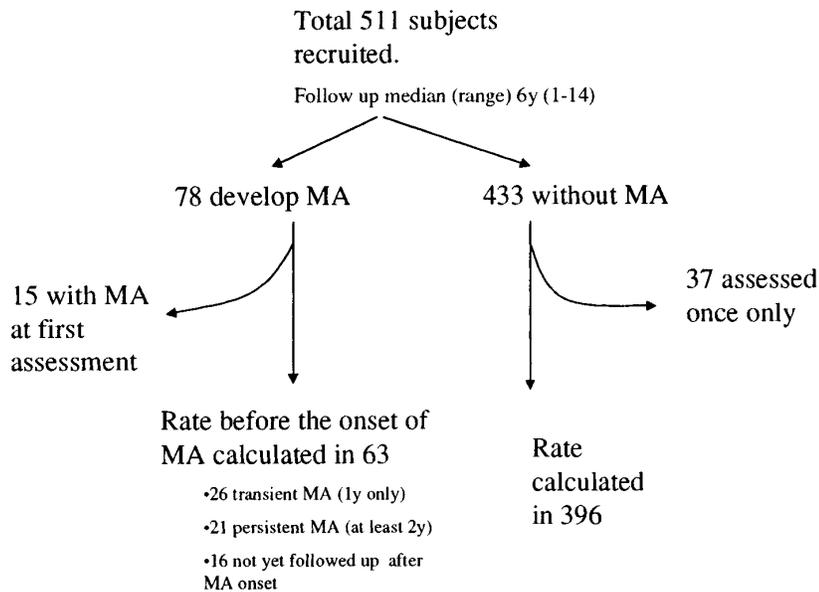
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Received for publication 18 April 2000 and accepted in revised form 16 August 2000.

**Abbreviations:** ACR, albumin-to-creatinine ratio; AER, albumin excretion rate; CV, coefficient of variation; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; IQ, interquartile; MA, microalbuminuria; ORPS, Oxford Regional Prospective Study.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.



**Figure 1**—Recruitment, duration of follow-up, and MA status. The persistent MA group included 16 subjects with MA for 2 consecutive years that did not regress, 4 subjects with MA that regressed after 2 consecutive years, and 1 subject with MA at more than two nonconsecutive annual assessments.

surement of the albumin-to-creatinine ratio (ACR). At 5 and 10 years after diagnosis, three timed overnight urine samples were collected for the estimation of the albumin excretion rate (AER). Urine specimens collected within the first 12 months after diagnosis were excluded from the analysis. In this article the “baseline” ACR was defined as the first determination of the ACR within 1–2.5 years from diagnosis.

The relationship between ACR and AER was determined in a previous study (3). MA was defined as the ACR equivalent to a level between 20 and 200 µg/min in two of three consecutive specimens (lower cutoff, males: ACR 3.5 mg/mmol; females: ACR 4.0 mg/mmol) (3). MA was defined as persistent if it was present for at least 2 consecutive years and transient if it was not present the year after first being detected (i.e., if it had regressed).

Urine albumin and creatinine were measured centrally as described in a previous study (3). Albumin was measured by a double-antibody enzyme-linked immunosorbent assay method with an interassay coefficient of variation (CV) of 12% at 1.5 mg/l and 10% at 16 mg/l. Creatinine was measured using the modified Jaffe method with a CV of 2% at 2.2 mmol/l (3). Longitudinal quality control data and improvements in methodology that were incorporated during the course of the study have been reported elsewhere (3).

HbA<sub>1c</sub> was also measured centrally (normal range 4.4–6.3%). An electrophoretic method was replaced with a high-performance liquid chromatography (HPLC) method in 1992. Longitudinal comparability of methods and quality control procedures have been described elsewhere (3). The HPLC method was comparable with that of the Diabetes Control and Complications Trial reference laboratory (3). The between-batch CV was 3.5% at a level of 5.6% and 2.2% at a level of 10.1%.

**Statistical methods**

The rate of increase of the ACR was estimated using the median ACR of all available specimens at each annual assessment. A plot of the ACR across the duration (in years) of diabetes was best represented by a log-linear curve. Thus, the rate of increase of the ACR in each subject was estimated as the slope (β) of the linear equation fitted on the logarithm of the ACR and the duration of diabetes (the independent variable):

$$\text{Log[ACR]}_n = \beta \cdot t_n + c$$

where  $t_n$  is years of duration of diabetes at the annual assessment ( $n$ ), and  $c$  is the intercept that represents the median ACR just before the onset of diabetes.

Non-normally distributed data were compared using the Mann-Whitney  $U$  test for independent samples, the Wilcoxon

signed-rank test for paired data, and the Spearman rank correlation coefficient. Pearson’s product moment correlation coefficient and Student’s  $t$  test (for independent samples) were used for normally distributed data. The median interquartile ranges are reported unless otherwise specified. Statistical significance was defined as  $P < 0.05$ . We used SPSS 8.0 for Windows.

**RESULTS** — The ACR was assessed for a median of 4 (range 1–11) annual assessments in control subjects and 3 (range 1–9) in cases before the onset of MA. In 54% of the subjects, ≥80% of the intended urine specimens were collected (i.e., they were collected in 5 of the median 6 years of follow-up). In 94% of subjects, ≥50% of the intended urine specimens were collected (i.e., ≥3 of the 6 years).

Figure 1 summarizes recruitment, duration of follow-up, and MA status. In our study, 78 subjects developed MA (designated cases), and 15 subjects had MA at the first assessment. These subjects were generally pubescent and had higher levels of HbA<sub>1c</sub> at the first assessment than the other subjects (Table 1). After excluding the 15 subjects with MA at the first assessment and 37 non-MA subjects who were only assessed once, 459 subjects remained.

The ACR data at baseline, defined as the first assessment between 1 and 2.5 years after diagnosis, were available for 355 of the 459 subjects. The baseline ACR (median [IQ range]) was significantly higher in the case subjects with MA compared with normoalbuminuric control subjects (1.0 mg/mmol [0.6–2.1],  $n = 52$ , vs. 0.8 mg/mmol [0.6–1.2],  $n = 303$ ;  $P = 0.02$ ). This is shown in Table 1 and includes both subjects assessed only once and those with MA at the first assessment. The baseline ACR was similar in subjects with transient and persistent MA (Table 1). The ACR at baseline was correlated with HbA<sub>1c</sub> at baseline ( $r = 0.34$ ,  $P < 0.001$ ,  $n = 318$ ).

MA was persistent in 21 cases, transient in 26 cases, and could not be evaluated in 16 cases (Fig. 1). The median rate of increase of the ACR was significantly higher in cases compared with control subjects (70% per year [37–149],  $n = 63$ , vs. 1% per year [–9 to 13],  $n = 396$ ;  $P < 0.001$ ). However, the rates were similar in the transient and persistent MA subgroups (Fig. 2). Furthermore, the ACR at the first appearance of MA was similar in the transient group compared with the persistent MA subgroup (Table 1).

**Table 1**—Sex, maximum duration of follow-up, mean HbA<sub>1c</sub>, and age-group at diagnosis of subjects with normoalbuminuria and MA, divided into subgroups according to the natural history of MA

	Normoalbuminuria	MA at first assessment	Transient MA*	Persistent MA
n (% male)	396 (57)	15 (47)	26 (46)	21 (43)
Diabetes duration at latest assessment (years)	6 (4–8)	2 (1–4)	5 (4–8)	7 (5–9)
Mean overall HbA <sub>1c</sub> (%)	9.6 (8.6–10.6)	11.7 (8.1–12.1)	9.6 (9.1–10.8)	10.8† (9.5–12.7)
Measurements (n)	394	11	26	21
Mean pubertal HbA <sub>1c</sub> (%) after age 11 years	9.8 (8.7–10.9)	10.9‡ (10.0–12.2)	9.8 (9.2–10.7)	11.7‡§ (9.9–12.9)
Measurements (n)	355	12	26	21
ACR at baseline (mg/mmol)	0.8 (0.6–1.2)	—	0.7 (0.4–1.2)	1.0  ¶ (0.6–1.5)
Measurements (n)	303	—	17	14
ACR at first appearance of MA (mg/mmol)	—	—	5.7 (3.9–9.6)	7.0   (4.6–11.4)
Measurements (n)	—	—	26	21
Age-group at diagnosis (%)				
<5 years	18	27	19	10
5–11 years	46	13	58	52
>11 years	36	60	23	38

Data are median (IQ range), unless otherwise indicated. \*Data on 16 subjects with unknown course of MA are not reported; †P = 0.029 vs. normoalbuminuric group; ‡P = 0.009 vs. transient group; §P < 0.001 vs. normoalbuminuric group; ||not statistically significant vs. transient group; ¶P = 0.02 vs. normoalbuminuric group.

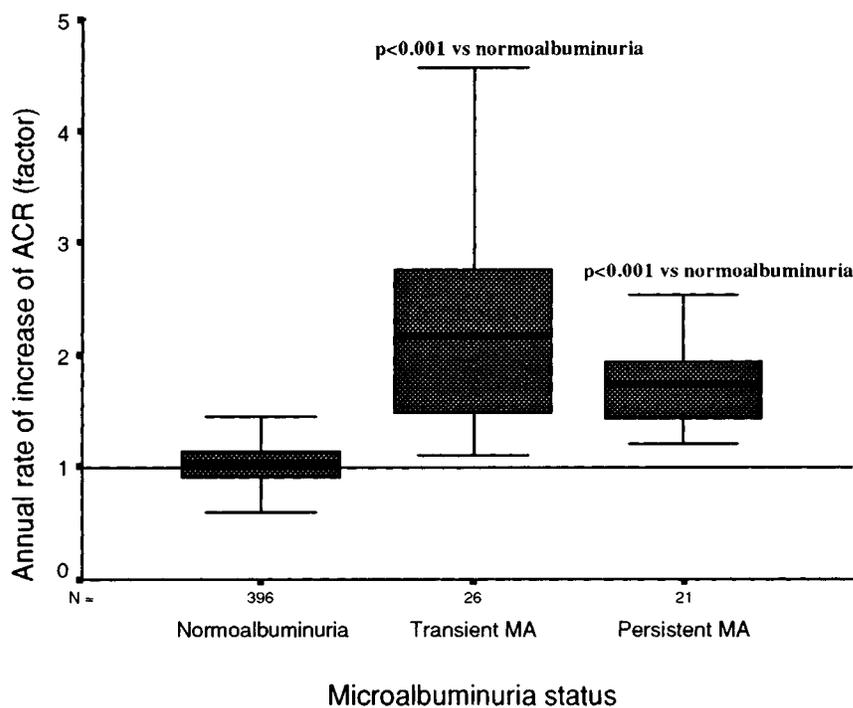
To assess the effects of puberty on the rate of change of the ACR, ages >11.0 and ≤11.0 years were used as proxies for puberty and prepuberty, respectively. The rate of change of the ACR during prepuberty before the onset of MA could be calculated in 16 cases in subjects aged ≤11 years at diagnosis, and the rate during puberty was calculated in 22 cases in subjects aged >11 years at diagnosis. The ACR increased at a significantly higher rate after the onset of puberty compared with prepuberty (80% per year [47–150], n = 22, vs. 26% per year [–7 to 76], n = 16; P = 0.003). In 30 subjects with MA, a rate of change could be calculated after the onset of MA when the first measurement of MA was excluded. Within this group, the median rate was significantly lower in the years after the onset of MA when compared with the years before (2% per year [–24 to 46] vs. 88% per year [78–174], n = 30; P < 0.001). The median age of these 30 subjects in the years after the first appearance of MA was 18 years (range 15–20).

The mean HbA<sub>1c</sub> level was calculated from all available measurements up to the first onset of MA or last follow-up. No HbA<sub>1c</sub> data were available for six subjects. Overall, the mean HbA<sub>1c</sub> level was significantly higher in the study's cases than in the control subjects (mean ± SD: 10.4 ± 2.0%, n = 63, vs. 9.7 ± 1.6%, n = 394; mean difference [95% CI]: 0.7% [0.3–1.2], P = 0.001). However, when compared with the non-MA group, the mean HbA<sub>1c</sub> level after

the onset of puberty was higher in both subjects in whom MA was detected at the first assessment and in those with persistent MA, but not in subjects with transient MA (Table 1). The mean HbA<sub>1c</sub> level after the onset of puberty was weakly correlated with the rate of change of the ACR (r = 0.11, P = 0.024, n = 418).

**CONCLUSIONS**—ORPS, a highly representative population-based study with longitudinal urine data collection that was on average 80% complete, represents the largest incidence cohort of children with type 1 diabetes reported to date (3).

The ACR increased by a median of 70% per year, for a median of 6 years from



**Figure 2**—Annual rate of change of the ACR in subgroups of MA. The median, IQ range, and 95% CI are shown. The reference line represents no change.

diagnosis, in subjects who developed MA. By contrast, the rate of change of the ACR was only 1% per year in subjects who did not develop MA. These are the first data to clearly indicate that subjects at risk of diabetic nephropathy can be identified within the first few years after diagnosis—and before the onset of MA—by sequential annual measurements of the ACR.

We investigated the effects of puberty using age 11 years as a proxy for puberty, as Tanner staging was not available. The rate of increase of the ACR in childhood was lower in the prepubertal years (26% per year) than in the pubertal years (80% per year). Similar observations were reported from a smaller (74 subjects) 3-year prospective study using Tanner puberty staging (9). Data from that study was also compatible with a higher rate of increase in the ACR during puberty when compared with postpuberty (9). Interestingly, in our study, the median age of the subjects in the years after the onset of MA was 18 years (i.e., postpubertal), and the median rate of increase of the ACR was low, only 2% per year.

Our findings are consistent with two previous smaller studies that reported a lower annual rate of increase of ~40% per year in 13 (7) and 15 adults with type 1 diabetes (10) who developed MA. The higher rate of change of the ACR in our study when compared with the aforementioned studies in adults (7,10) may relate to the effects of puberty and differences in glycemic control between the study populations. These studies in adults (7,10) also included measurements taken before and after the onset of MA in their calculations of rates; it is not known how much of the data represented the years before the onset of MA (7,10). In our study, the lower rates of increase in the ACR after MA onset may relate to the regression of MA.

MA is known to regress in some patients, and in our study, MA regressed after the first year in 26 subjects (55%) and after the second year in 5 more subjects. A small study reported that in 6 of 10 adolescent females with type 1 diabetes, the number of years before regression of MA varied according to their age at the end of puberty (11). This finding is consistent with a larger prospective study of 164 children, which found that puberty is a risk factor for MA (12). However, the duration and growth effects of puberty may vary between individuals (13). This probably reflects differences in levels of hormones related to growth and sex, namely growth hormone

IGF-1 and sex steroids (14). Therefore, the length of time that MA persists before regression may relate to differences in the phenotype of puberty. Furthermore, puberty and HbA<sub>1c</sub> are independently associated with increased risk of MA (3). In our study, the mean HbA<sub>1c</sub> level was lower in subjects who developed transient MA when compared with those in whom MA was persistent. These data suggest that the increase in risk of MA during puberty may be transient in some subjects protected by lower HbA<sub>1c</sub> levels. We observed a weak ( $r = 0.1$ ) association between HbA<sub>1c</sub> and the rate of increase of the ACR before the onset of MA, perhaps because the effect of puberty was dominant.

Renal biopsies were not examined in our study, which is perhaps a limitation of this report. Other studies of renal biopsies suggest that levels of urine albumin excretion below the cutoff for MA increase concomitantly with histological changes in renal morphology that are compatible with early diabetic renal injury (15,16). These data, together with our study, suggest that the rate of change of the ACR is a biochemical estimate of the rate of renal morphological changes related to diabetic nephropathy.

Our results suggest that there was a similar degree of renal injury in the transient MA group compared with the persistent MA group, because the rate of increase of the ACR was similar in the transient and persistent MA subgroups. Furthermore, there was no significant difference in the levels of the ACR at the first appearance of MA. The level of HbA<sub>1c</sub> was lower by ~2% in the transient compared with the persistent MA group. Therefore, during puberty, transient renal injury may occur in individuals protected by a lower HbA<sub>1c</sub> level. Genetic susceptibility to nephropathy is thought to be important in the 30–40% of subjects with type 1 diabetes who may be at risk of developing this complication (17). The hypothesis that puberty may be a “stress test” for the detection of incipient renal injury (11) may be extended by our observations that susceptibility to diabetic renal injury can be detected by a progressive rise of the ACR (below the level of MA) during puberty. Subjects diagnosed with type 1 diabetes before or during puberty are at a higher risk of nephropathy when compared with subjects diagnosed after puberty (18). It is possible that accumulated renal damage resulting from transient injury during puberty may be an important determinant of future nephropathy risk.

In conclusion, the development of MA is associated with a higher ACR level between 1 and 2.5 years after diagnosis. A rise in the ACR before the onset of MA can be detected within the first 6 years after diagnosis during adolescence. Both susceptibility to MA and the rate of rise of ACR are related to HbA<sub>1c</sub>, although puberty may be more important in determining the rate of progression of diabetic renal injury. Further follow-up of this cohort is needed to determine whether the rate of increase of the ACR may be a risk marker for diabetic nephropathy or cardiovascular disease.

**Acknowledgments**— The ORPS is funded by the British Diabetic Association.

We thank Sue Standing for HbA<sub>1c</sub> data conversion. We acknowledge the continuing help and support from the children and their families; the study field workers, Vanessa Carter and Emma Moore (previously Lin Barnetson, Judy Downey, Ann Kingsmill Moore, and Fran Traynor); the laboratory assistance of Angie Watts, Dot Harris, Tina Carroll, and Charles Turner; the Barts-Oxford Study fieldworkers; and pediatricians, physicians, and diabetes nurse specialists in the Oxford region. We thank Pat Yudkin, University Lecturer in Medical Statistics (Department Primary Health Care, Institute of Health Sciences, Oxford) for statistical advice.

**APPENDIX**— Members of the ORPS Steering Committee: Prof. D.B. Dunger, Dr. R.N. Dalton, Prof. J. Fuller, Prof. E.A.M. Gale, Prof. H. Keen, Dr. M. Murphy, Dr. H.A.W. Neil, Dr. C.J. Schultz, Dr. R.J. Young, and T. Konopelska-Bahu. Members of the Oxford Regional Prospective Study Group: Drs. R.A.F. Bell and A. Taylor, Horton General Hospital, Banbury; Drs. A. Mukhtar, B.P. O'Malley, B.R. Silk, and E.H. Smith, Kettering District Hospital; Drs. R.D.M. Scott, King Edward VII Hospital, Windsor; Drs. F.M. Ackland, C.J. Fox, and N.K. Griffin, Northampton General Hospital; Drs. N. Mann, H. Simpson, P. Cove Smith, and M. Pollitzer, Royal Berkshire Hospital, Reading; Drs. R.S. Brown and A.H. Knight, Stoke Mandeville Hospital, Aylesbury; Drs. J.M. Cowen and J.C. Pearce, Wexham Park Hospital, Slough. The Box Study was coordinated by P.J. Bingley, Southmead Hospital, Bristol.

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