Borderline Increases in Albumin Excretion Rate and the Relation to Glycemic Control in Subjects with Type I Diabetes

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We evaluated "borderline" increases in overnight albumin excretion rates (AERs)—i.e., those between the upper 95th percentile of normal (7.6 µg/min) and the lowest value currently considered predictive of nephropathy (30 µg/min)—to determine their importance and to see whether glucose control influenced subsequent changes in the "borderline" AER values. Between 1985 and 1990, we studied 190 subjects with insulin-dependent diabetes mellitus (Type I), analyzing a mean of 6.5 timed overnight urine samples collected per subject. Above-normal AERs were associated with a significantly (by ANOVA) higher mean age (P = 0.03), longer duration of diabetes (P = 0.0002), and greater mean glycohemoglobin values (P = 0.002). The transition rate between borderline and abnormal AERs was significantly higher (P < 0.0001, chi-square test) than the direct transition rate between normal and abnormal AERs, thus showing the borderline AER to be a definite intermediate stage. Good and poor glucose control were clearly associated with improvement and worsening, respectively, of the borderline AER values (P = 0.032, chi-square test of trend). More attention to borderline AER values is clearly indicated.

Additional Keyphrases: glycohemoglobin · nephropathy · urine · Markov statistical model

Renal failure secondary to diabetic nephropathy is one of the leading causes of morbidity and mortality among subjects with diabetes mellitus. An overnight albumin excretion rate (AER) of >30 and <140 µg/min reliably predicts the future development of nephrosis (>0.5 g of albumin excreted per 24 h) (1). The AER lower bound of 30 µg/min was retrospectively set as a value that would allow accurate prediction of nephropathy before normal control values for an overnight AER had been determined (1). Recognition of this stage of AER (30–140 µg/min) is sometimes too late to allow successful medical intervention. Renal biopsies have shown changes to sometimes be present even when the AER is <30 µg/min (2). Several investigators have used a lower bound of 15 µg/min to predict nephropathy (3–5), although the importance of lower AER values as an early marker of renal damage needs further evaluation. It is hoped that markers that allow earlier detection of a trend toward nephropathy will allow earlier efforts to improve glucose control—alone, or with medications—to retard the progression of early renal damage.

The 95th percentile of normal for an overnight AER is now known to be 7.6 µg/min (6). Because albumin is a relatively large molecule with only small amounts normally excreted in the resting state from normal kidneys, probably any increase in AER (>7.6 µg/min) indicates early renal damage. Our goal was to determine whether there is a "borderline" stage of AER that differs from a normal or an abnormal AER, and to determine the influence of glycemic control on borderline AER values.

Materials and Methods

Subjects
Subjects diagnosed to have Type I (insulin-dependent) diabetes before age 21 years (n = 228) collected a mean of 4.9 timed overnight urine samples for AER determinations, bringing in samples on at least two separate clinic visits. All subjects followed in our diabetes clinic who are 14 years or older and who have had insulin-dependent diabetes mellitus for at least five years are encouraged to participate in our eye–kidney clinic. Approximately 95% of eligible subjects attend this clinic. All subjects are asked to bring in two timed overnight urine samples once yearly, although sometimes only one (or none) was provided. The subjects of this report collected at least two initial and two final timed overnight urine samples at least one year apart; there were no other exclusion criteria for the study. These 190 subjects collected a mean of 6.5 overnight urines each.

Albumin excretion (AER) was expressed in micrograms per minute. The lower detection limit of the albumin assay (RIA Albumin Double Antibody Kit; Diagnostic Products Corp., Los Angeles, CA) was improved by routinely including a 2.5 mg/L sample in the standard curve. The lower limit for AER detection in our laboratory is 0.5 µg/min and the interassay and intra-assay coefficients of variation are both <4% (6). An abnormal AER is defined as a mean of two or more AER values that exceeds 30 µg/min, a value that has been shown to be "a good predictor of clinical proteinuria, which is a known precursor of renal failure" (1).

Forty-one healthy young nondiabetic subjects volunteered to do overnight urine collections for AER determinations. As reported elsewhere, their 95th percentile of normal for overnight AER was 7.6 µg/min (6). A borderline AER is defined as a mean of two or more values between 7.6 and 30.0 µg/min; a normal AER is defined as the mean of two or more values <7.6 µg/min. We set these definitions for grouping prospectively, before inspection and analysis of the data.
Among 190 subjects, 632 urine samples were available from two consecutive days. All urine samples for days 1 and 2 were classified into the three above categories. A 3 x 3 frequency table was used to obtain the degree of concordance between the two days' AER values. By the measure of association Gamma, 90% of the samples were concordant for their second sample.

AER was measured either in fresh urine samples or after storage for less than two weeks at 4°C. This is important because freezing the sample lowers the AER results, particularly for samples in the borderline range (6). Subjects are asked not to collect the samples during menses or if they had performed heavy exercise in the 4 h before beginning the collection. All samples were screened for ketonuria and leukocytes and excluded if found positive (6). All AER determinations were done by the same method during the study period. The mean of the two AER values was used for analysis of data.

All glycohemoglobin (HbA1c) proportions were determined with the use of ion-exchange resin (Fast Hemoglobin Test System; Isolab, Akron, OH), for which normal values were between 6.3% and 8.2%. Normal values have remained the same from 1979 to the present, even though initially the labile fraction was removed by overnight saline incubation but now is removed by a reaction with an aldime eliminator. The interassay coefficient of variation is 6%. When more than one HbA1c value was available during the 12 months before determination of AER, the mean of all values was used for data analysis. A change in HbA1c values was defined as improved or worsened only if the change between the initial value and the final mean value was >10%. The HbA1c was designated as the "same" if the change between the two values was ±10%. We arbitrarily consider an HbA1c <1.3 x the upper limit of normal as a "good" value (<10.7% for our method) and a value >1.5 x normal (12.3%) as indicative of "poor" glucose control. In previous work (7) we have discussed the rationale for use of these classifications in relation to the probability of complications.

Statistical Methods

A special program for the Markov model (8, 9) was used for analysis of transition rates and for describing the rate of progression and regression of AERs. This model was also used to predict the reliability of borderline AER values as an earlier marker of kidney disease.

The probability matrix was calculated by using a computerized formula: \( P = e^Q \) where \( Q \) is the (3 x 3) matrix of transition rates (8).

The Markov model using the Markov process, as recently described by Kay (8), is an extension of Markov chains using continuous time. This model allows estimation of the transition probability matrix when the data are collected at different intervals. The Markov process was used to confirm the estimations of the transition matrix initially calculated by using discrete times (Markov chains).

We used the SAS program (10) for the remainder of the data analyses. Statistical analyses included the use of the chi-square test, Student's t-test, and analysis of variance (ANOVA). When multiple comparisons were made, the individual significance values were appropriately modified by the method of Bonferroni (11).

Results

The subjects collected a minimum of two initial timed overnight urine samples for determination of AER and a minimum of two additional samples at least one year later. The mean time between the collection of the initial and the final urine samples was 2.1 years (range, one to four years). The mean age, duration of diabetes, and gender distribution for the 190 subjects are shown in Table 1.

Among the 60 subjects with initial "borderline" AERs (Table 1), 52 had rates between 7.6 and 20.0 μg/min and eight had rates between 20.1 and 30.0 μg/min. Six of the eight (75%) and nine of the 52 (17%) have since progressed to having AERs consistently >30 μg/min.

The subjects progressing to an abnormal AER are shown in Figure 1 with the time of their AER (mean of two or more samples) first exceeding 30 μg/min arbitrarily set as time zero. For the subjects whose AER values remained normal, time zero represents their last overnight urine collections. The subjects who developed an abnormal AER had significantly higher overnight AER values in the preceding three years than did the group of subjects whose values remained normal until their last visit (P <0.05; Bonferroni's multiple t-tests). Moreover, no subject who initially had a normal AER progressed to having an abnormal value without first passing through a borderline AER value.

Three of the 38 subjects showed reversal from an abnormal AER (>30 μg/min) to a normal or borderline

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Table 1. Initial Demographic Information

<table>
<thead>
<tr>
<th>AER, μg/min</th>
<th>Gender,</th>
<th>Age, years</th>
<th>Duration of diabetes, years</th>
<th>HbA1c, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (&lt;7.6)</td>
<td>92</td>
<td>43/49</td>
<td>17.7 ± 0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.1 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Borderline (7.6-30)</td>
<td>60</td>
<td>35/25</td>
<td>18.1 ± 0.35</td>
<td>10.3 ± 0.54</td>
</tr>
<tr>
<td>Above normal (&gt;30)</td>
<td>38</td>
<td>18/20</td>
<td>19.4 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.2 ± 0.87&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>P (by ANOVA)</td>
<td>N.S.</td>
<td>0.03</td>
<td>0.0002</td>
<td>0.0022</td>
</tr>
</tbody>
</table>

<sup>a</sup> The mean of all values in the year preceding the first borderline or above-normal AER.

<sup>b</sup> These values in each column are significantly different by Bonferroni's multiple t test at P <0.05.

<sup>c</sup> Not significant (by chi-square test).

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CLINICAL CHEMISTRY, Vol. 37, No. 12, 1991 2049
AER. All three improved their mean HbA₁ values by >10% (mean improvement, 21%).

Eight of the 27 subjects (30%) with improvement in their borderline AER had a decline (by >10%) in their HbA₁ results, whereas three had an increase (Table 2). In addition, 10 others had HbA₁ values in the "good" range (<1.3 x normal), both at the time of the initial borderline increased AER and upon follow-up, when the AER was normal. These 10 subjects are in the "same" group in Table 2. Thus, 18 of the 27 subjects (66%) showing improvement in their AER either had a decline in HbA₁ (>10%) or had an HbA₁ value consistently in the "good" range. The alterations in two variables (HbA₁ and AER) during the study period are significantly associated (P = 0.032, chi-square test of trend). The statistic Gamma for this association is 0.4, showing the important degree of concordance.

Seven of the 14 subjects (50%) whose borderline AER worsened also had an increase in their HbA₁; three others showed improvement. Two others had values that remained within 10% of their initial HbA₁ but their mean values for initial and final HbA₁ proportions were both >1.5 x normal (and classified in our "poor" control category). Thus, nine of the 14 subjects (64%) who had AERs that went from borderline to abnormal either had an increase in their HbA₁ or had an HbA₁ value consistently in the poor range. AERs during the period of follow-up did not improve or worsen in 18 other subjects with borderline AERs. Eight of these 18 (45%) maintained the same HbA₁ values, while four subjects had decreasing values and six had increasing ones.

The mean (±SEM) HbA₁ in the year preceding the final AER testing was 10.9% (0.32%) for subjects who had AER values that improved from borderline to normal, 11.8% (0.53%) for those who continued to have borderline values, and 12.9% (0.60%) for those who had values that progressed from borderline to abnormal (P = 0.015; ANOVA).

Markov's model was used to describe the transition rates between the three groups: normal, borderline, and abnormal (Figure 2). A total of 328 transitions were available for 190 subjects. These 190 subjects collected a mean of 4.9 overnight urine samples per person for determinations of AER. The model demonstrated that in four years, 15% of subjects would be predicted to have AERs that changed from normal to abnormal. Similarly, 30% of subjects with "borderline" AER values would be predicted to have abnormal values in four years. As summarized in Figure 2, the transition rate between normal and borderline AERs is 0.0243, indicating that it would take a mean of 33 months for an individual subject to progress from a normal to a borderline AER. The 95% confidence interval for this change is 0.0153–0.033. The transition rate for progression from a borderline to an abnormal AER is 0.0165, indicating that it would take a mean of 18.5 months to progress from a borderline to an abnormal AER. The 95% confidence interval for this change is 0.0084–0.0243. The regression transition rate from a borderline to a normal AER is 0.0588, which is 3.5 times greater than that for a progression from a borderline to an abnormal AER. These data suggest that the borderline AER is an "unstable" stage before the development of a

![Figure 2](https://example.com/figure2.png)

Fig. 2. Transition rates (±SEM) between normal, borderline, and abnormal AER values by the Markov model (8, 9) for 190 subjects with insulin-dependent diabetes mellitus

The transition rate from a borderline to an abnormal AER was significantly higher than from a normal to an abnormal AER (P <0.001, chi-square test). The model demonstrates that in four years the AER values of 15% of the subjects would be predicted to change from normal to abnormal; the AER values in 30% of the subjects would be predicted to change from borderline to abnormal.
consistently abnormal AER. However, because the transition rate between borderline and abnormal AERs was significantly higher \( (P < 0.0001, \text{chi-square test}) \) than the direct transition rate between normal and abnormal, the borderline AER is a definite intermediate stage.

Discussion

This study shows that borderline overnight AER values (>95th percentile of normal but <30 \( \mu \text{g/min} \)) are an important intermediary between normal and abnormal values and should not be ignored. Using the Markov model, we showed that borderline AER values predicted the development of an abnormal AER (>30 \( \mu \text{g/min} \)) in about one-third of subjects within four years. All subjects who developed an abnormal AER during the course of this investigation first went through a period of having a borderline AER. This suggests that the process of renal damage is usually slow enough that the recommended annual screening \((12, 13)\) will detect the borderline AER state. The importance of these findings relies on the assumption that those who progress from a borderline AER to an AER >30 \( \mu \text{g/min} \) eventually develop macroalbuminuria or renal failure.

Mogensen and Schmitz \((14)\) reviewed the progression rates of microalbuminuria in diabetic renal disease and considered the clinical intervention trials that have evaluated the effect of metabolic control on preventing or retarding the renal disease of diabetes. They concluded that "metabolic control is likely to be of decisive importance, predominantly for the development of early glomerular lesions." The Oslo Microalbuminuria Study showed a reduction in urinary albumin excretion after four years of continuous subcutaneous insulin infusion \((15)\). The present study further highlights the importance of improving glycemic control at a definitive intermediate stage in an attempt to reverse early diabetic nephropathy. Of our subjects who progressed from having borderline to abnormal AERs, 64% also showed worsening of their HbA\(_1\) values or consistently poor glycemic control. In contrast, 18 of the 27 subjects (66%) who showed improvement in their borderline AER values had either a decrease in their HbA\(_1\) values or a "good" mean HbA\(_1\) in the year of final AER testing. Although the present study found an association between HbA\(_1\) proportions and AER, this does not of course prove a definitive causal relationship.

The cumulative incidences and the progression rates of early diabetic nephropathy have been described previously \((1, 14)\). However, the use of Markov's model takes into account each transition longitudinally for all subjects and calculates the lifetime risk of developing later stages of diabetic nephropathy. The model describes our present data, collected over four years. We have not attempted to predict final morbidity data, because we believe that predicting long-term probabilities as the cumulative incidence may not be appropriate. The model assumes that all patients have the potential of developing an abnormal AER. It is not necessarily valid to predict the percentage whose values will become abnormal.

Some have suggested that new tests are needed to detect early renal damage in subjects with diabetes. Perhaps better application of tests already available may be all that is needed. Slight ("micro")albuminuria is measured with a sensitive radioimmunoassay and nonfrozen timed urine collections \((6)\). The physiological variability in the test due to daytime exercise/orthostatic proteinuria \((16)\) is decreased by using overnight resting urine collections. These collections are relatively easy for patients because they do not have to collect urine during the day. Given the variability of AER values \((17, 18)\), two or more overnight urine collections are recommended at least once yearly for all pubertal subjects who have had insulin-dependent diabetes mellitus for five years or longer. If results of the two samples do not agree, collections should be repeated every six months until satisfactory results are obtained.

This study shows that the borderline AER represents an important intermediary stage at a time when reversal appears to be possible by improving glucose control. Further documentation of factors influencing the reversibility of borderline AERs will be important.

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References

14. Mogensen CE, Schmitz O. The diabetic kidney: from hyperfil-